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PESTICIDES IN PEOPLE

Epidemiology of Organochlorine Insecticides in the Adipose Tissue of Israelis [1]

M. Wassermann,[2] L. Tomatis,[3] Dora Wassermann,[2] N. E. Day,[3] Y. Groner,[2] S. Lazarovici,[2] Deborah Rosenfeld [2]

ABSTRACT

This paper reports the findings obtained in 1967-71 during an investigation of organochlorine insecticides (OCI) storage in the adipose tissue of Israelis.

Specimens of adipose tissue (307) collected during autopsy from Israelis who had no known occupational exposure to organochlorine insecticides were analyzed by the gas chromatographic method for organochlorine insecticides (DDT-derived material; alpha, beta, and gamma isomers of BHC, dieldrin, and heptachlor epoxide).

Findings indicate a positive age association for DDT-derived material stored in the adipose tissue of Israelis of both sexes. Males generally were found to store higher amounts of p,p'-DDT and total DDT than females.

Comparison of adipose tissue from stillborns with tissue from infants showed that DDT increased in the first months of postnatal life, but storage levels of BHC, dieldrin, and heptachlor epoxide remained approximately the same. DDT levels continued to rise with age levels, except for a slight decrease in the 24-through-44-year-olds. The highest levels of DDT were found in the age group of 70 and over; second highest were among 45-to-69-year-olds. These findings in Israel differ from the authors' earlier findings in South Africa, Thailand, Nigeria, and Brazil, which revealed the highest concentrations of OCI in the 24-through-44-year-olds.

A positive age association with DDT storage in all ages was observed in 1965 and 1967 surveys by the authors in people from Kenya and Israel, and by Davies and Milby in the nonwhite population of the USA.

In the countries studied, the storage level of DDT and derived material increases with age in the general population up to the age of 45, and either rises or falls after 45 years, depending on the country. This leads the authors to the opinion that the age group of 25 through 44 years may be the most suitable indicator of DDT storage levels in a community.

A positive relationship between p,p'-DDT and dieldrin storage was also noted.

Introduction

The presence of DDT and its metabolite DDE in the adipose tissue of the population of Israel was first demonstrated in a study carried out by Wassermann et al. (*1*) in 1963-64. They revealed that these chemicals were stored in the adipose tissue of people who had no known occupational exposure to the chemicals and suggested that the DDT storage process is influenced by individual factors such as sex and age.

A further study carried out by the same investigators in 1965-66 (*2*) ascertained that Israelis over 10 years of age stored higher amounts of DDT-derived material than did those under 10 years of age. Males over 10 stored higher amounts of DDT-derived materials than did females.

A study by Polishuk, Wassermann, et al. (*3*) on pregnant Israeli women indicated less storage of DDT-derived

[1] This research study was supported by the U.S. Department of Health, Education and Welfare, Public Health Service, National Communicable Disease Center, Atlanta, Ga., Research Grant No. BSS-CDC-IS-9, and by an agreement of the World Health Organization—International Agency for Research on Cancer.

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[3] World Health Organization—International Agency for Research on Cancer, Lyon, France.

material, BHC isomers, and dieldrin than in nonpregnant women of the same age. The organochlorine insecticides (OCI) found in the fat tissue of pregnant women were present also in the maternal and fetal blood in all cases studied. These findings suggested that, in pregnancy, the metabolism of organochlorine insecticides is enhanced and that they pass the placental barrier.

Data published up to the mid-sixties on the storage of DDT-derived material in the adipose tissue of people from various countries affirmed the concept of organochlorine insecticides as "current constituents of the human body" (*4*). Other studies revealed the impact exerted on OCI storage in man by individual factors such as age, sex, and race (*1, 2, 5-11*), by particular physiological states (*3, 12*), as well as by living and working conditions (*7, 13*).

Concern for the potential hazard to human health by organochlorine insecticide accumulation in the body increased following reports that OCI are potent hepatic microsomal inducers which may quantitatively alter the response to various drugs and toxic compounds as well as to naturally occurring substances in the animal body. This may lead to alteration of homeostasis of biochemical (endocrine, immunologic, etc.) processes (*3, 14-16*). Experimental evidence of the capacity of OCI to increase tumor incidence in laboratory animals was also reported (*17-19*).

Thus it became necessary to assess the size and trends of OCI storage in populations in general and to find which age group would be most useful for studying this storage phenomenon in various areas of the world.

This paper reports the findings of a study of OCI storage in Israelis and compares the data to those obtained by the same investigators in various populations in Africa, Asia, and South America in the framework of a program launched by the World Health Organization International Agency for Research on Cancer, Lyon, France.

Methods and Materials

A total of 307 samples of adipose tissue were collected from 1967 to 1969 during autopsy from the subcutaneous fat of the abdominal wall of persons who had no known occupational exposure to pesticides. The distribution of samples according to age and sex is shown in Table 1. Samples of 1-2 g of adipose tissue were collected in jars containing 10% formalin. Specimens of 500-mg adipose tissue were extracted three times with a total of 20 ml of petrol ether and cleaned by means of a Kontes Co-Distiller. The extract was reduced to 0.5 cc, from which 5-20 μl were injected into a Microtek MT-220 gas chromatograph equipped with dual electron-capture detector and strip chart recorder. A 6-ft U-shaped glass column packed with 5% QF-1 on 60-80 mesh chromoport xxx and a 4-ft U-shaped glass column packed with 10% SE-30 on 60-80 mesh chromoport xxx were used. A mixture of pure organochlorine insecticides was used as standard; the concentration of each compound was 0.1 ppm. Recovery was about 85% for the compounds identified in this study. The sensitivity of detection was 0.1 to 0.3 μg/kg wet weight for these compounds.

TABLE 1.—*Distribution of human adipose tissue samples by age and sex of subject*

AGE	MALES	FEMALES	TOTAL
Stillborns	26	18	44
0-11 months	22	18	40
5-24 years	38	23	61
25-44 years	29	24	53
45-69 years	32	31	63
70 and over	27	19	46
Total	174	133	307

Results and Discussion

In 307 samples of adipose tissue collected from Israelis who had no known occupational exposure to organochlorine insecticides, gas-chromatographic analysis revealed the presence of DDT-derived material; alpha, beta, and gamma isomers of BHC; dieldrin; and heptachlor epoxide.

It must be stressed that, as noted in previous studies (*20-23*), marked individual variations in storage levels were observed among individuals of the same sex and age group who were not occupationally exposed to OCI's. Similar individual variations were observed in experimental animals kept in experimental conditions in which all were exposed to the same level of DDT (*24*).

The broad spectrum of age groups and the relatively large number of cases investigated in this study enable us to follow up the dynamics of OCI storage in this population by age and sex.

Concentration of OCI in the adipose tissue of the stillborn group averaged: total DDT 0.7 ppm; total BHC 0.04 ppm; dieldrin 0.02 ppm; and traces of heptachlor epoxide below 0.01 ppm, thus indicating the accumulation of these compounds during fetal life (Tables 2-4).

The first months of life in the external environment led to an increased DDT storage. In the age group under 11 months, total DDT averaged 5.8 ppm; the storage level of BHC, dieldrin, and heptachlor epoxide remained about the same as in stillborn. DDE averaged 51.7% of total DDT-derived material. The mean total *p,p'*-DDT was 5.7 ppm and the mean total *o,p'*-DDT was 0.09 ppm. There was a significant difference in the storage of *p,p'*-DDT and total DDT between the stillborn and the 0-through-11-month age group ($p < 0.01$).

TABLE 2.—*Concentration (ppm) of organochlorine insecticides in adipose tissue of Israeli males*

Compound		Age group and number of cases					
		Stillborns 26	0-11 mos. 22	5-24 yr. 38	25-44 yr. 23	45-63 yr. 32	70+ yr. 27
p,p'-DDT	Range	0.0030 — 1.2500	0.0940 — 5.0000	0.4444 —23.3330	0.0550 —11.1200	0.2190 —24.0000	0.3570 —34.2850
	Mean ± SD	0.2367 ± 0.3054	2.6566 ± 1.7438	4.1500 ± 4.5308	3.2841 ± 3.0574	5.8968 ± 5.4581	7.1626 ± 8.3018
p,p'-DDD	Range	<0.0001 — 0.0428	0.0028 — 0.7140	0.0555 — 3.0440	0.0258 — 2.0690	0.0920 —14.1025	0.1085 — 7.0450
	Mean ± SD	0.0134 ± 0.0141	0.1913 ± 0.5935	0.8606 ± 0.6006	0.8081 ± 0.6722	1.5722 ± 2.4413	1.8190 ± 1.6826
p,p'-DDE	Range	0.0175 — 1.7410	0.0860 — 8.7655	0.3194 —17.9440	0.1050 —55.0310	1.4100 —20.6920	2.1110 —31.2810
	Mean ± SD	0.5329 ± 0.1975	2.2101 ± 2.5431	9.8255 ± 8.8706	10.3300 ±10.4120	10.2741 ± 4.6160	10.4486 ± 6.8228
o,p'-DDT DDD	Range	<0.0001 — 0.0260	<0.0001 — 0.1100	0.0040 — 1.1250	0.0098 — 2.7170	0.0263 — 4.4740	0.0180 — 0.9800
	Mean ± SD	0.0100 ± 0.0100	0.0130 ± 0.0283	0.1472 ± 0.1794	0.2340 ± 0.4874	0.3673 ± 0.7583	0.2381 ± 0.1889
o,p'-DDE	Range	<0.0001 — 0.0250	<0.0001 — 0.0940	0.0120 — 0.0910	0.0028 — 0.6110	0.0120 — 0.8850	0.0070 — 0.2080
	Mean ± SD	0.0090 ± 0.0584	0.0164 ± 0.0173	0.0594 ± 0.0663	0.0872 ± 0.1349	0.0949 ± 0.5874	0.0532 ± 0.0424
Total *p,p'*-DDT	Range	0.0525 — 3.1209	1.1288 —18.6333	0.8557 —36.6056	0.9380 —67.9005	1.8817 —43.9410	3.2243 —70.4700
	Mean ± SD	0.7963 ± 0.8261	5.3101 ± 4.3301	15.8348 ±13.3770	15.6003 ±13.9170	18.9144 ± 9.8861	20.6214 ±15.8210
Total *o,p'*-DDT	Range	0.0043 — 0.0449	<0.0001 — 0.2147	0.0853 — 1.1384	0.0129 — 2.3061	0.0486 — 4.6292	0.0369 — 1.0335
	Mean ± SD	0.0201 ± 0.0100	0.0341 ± 0.0480	0.2137 ± 0.1838	0.3312 ± 0.5184	0.4731 ± 0.7942	0.2974 ± 0.2100
Total DDT	Range	0.0671 — 3.1359	1.1484 —18.8480	0.9471 —38.7134	0.9966 —68.1350	2.0797 —48.5702	3.3065 —71.1441
	Mean ± SD	0.8164 ± 0.8240	5.3435 ± 4.0977	16.0482 ±13.4530	15.9315 ±13.9290	19.3874 ±10.3620	20.9188 ±15.9130
α-BHC	Range	<0.0001 — 0.0458	0.0027 — 0.0580	0.0100 — 0.1240	0.0053 — 0.6200	0.0050 — 0.1800	0.0045 — 0.0886
	Mean ± SD	0.0091 ± 0.0099	0.0091 ± 0.0349	0.0298 ± 0.0245	0.0445 ± 0.1136	0.0285 ± 0.0332	0.0191 ± 0.0141
β-BHC	Range	<0.0001 — 0.1150	0.0021 — 1.0580	0.0259 — 2.0010	0.0054 — 2.9350	0.0301 — 4.7760	0.0500 — 2.9470
	Mean ± SD	0.0140 ± 0.2236	0.0721 ± 0.2168	0.2818 ± 0.4689	0.4468 ± 0.7866	0.5010 ± 0.8802	0.5408 ± 0.8004
γ-BHC	Range	<0.001 — 0.0777	0.0016 — 0.1090	0.0010 — 3.9170	0.0010 —12.1430	0.0010 — 0.2337	0.0010 — 1.2876
	Mean ± SD	0.0130 ± 0.1732	0.0198 ± 0.0224	0.1343 ± 0.6263	0.4365 ± 2.2124	0.0205 ± 0.0424	0.0735 ± 0.2417
Total BHC	Range	0.0054 — 0.1201	0.0123 — 1.0810	0.0492 — 4.3530	0.0232 —12.7010	0.0541 — 4.7980	0.0600 — 3.0230
	Mean ± SD	0.0361 ± 0.0283	0.1009 ± 0.2191	0.3987 ± 0.4498	0.5074 ± 0.8031	0.5500 ± 0.8739	0.6334 ± 0.8101
Dieldrin	Range	0.0003 — 0.1180	<0.0001 — 0.0940	0.0116 — 0.2100	0.0066 — 0.4660	0.0278 — 0.8970	0.0140 — 0.8020
	Mean ± SD	0.0305 ± 0.0283	0.0183 ± 0.0224	0.1070 ± 0.1311	0.1364 ± 0.1229	0.1899 ± 0.1752	0.1630 ± 0.7182
H. Epoxide	Range	<0.0001 — 0.0102	<0.0001 — 0.0120	0.0001 — 0.0480	<0.0001 — 0.1320	<0.0001 — 0.1180	<0.0001 — 0.0770
	Mean ± SD	0.0030 ± 0.0028	0.0007 ± 0.0025	0.0211 ± 0.0412	0.0205 ± 0.0316	0.0251 ± 0.0192	0.0102 ± 0.0027

TABLE 3.—*Concentration (ppm) of organochlorine insecticides in adipose tissue of Israeli females*

Compound		Age group and number of cases					
		Stillborns 18	0-11 mos. 18	5-24 yr. 23	25-44 yr. 24	45-63 yr. 31	70+ yr. 13
p,p'-DDT	Range	0.0028 — 0.3480	<0.0001 — 1.7850	0.2940 — 7.2910	0.0042 — 7.5100	0.0333 —11.3540	0.5140 —12.7010
	Mean ± SD	0.1040 ± 0.0806	2.1533 ± 2.6008	3.4744 ± 3.0769	2.6009 ± 2.2226	4.4725 ± 2.7399	3.6708 ± 3.0170
p,p'-DDD	Range	<0.0001 — 0.1125	<0.0001 — 0.1666	0.1142 — 1.5278	0.0091 — 2.1210	0.0709 — 4.3478	0.1270 — 2.9166
	Mean ± SD	0.0146 ± 0.0265	0.0192 ± 0.0400	1.0508 ± 1.8711	0.6896 ± 0.5036	1.0529 ± 0.9342	0.9769 ± 0.6058
p,p'-DDE	Range	0.0242 — 1.3131	0.1750 —15.4900	0.5367 —21.2698	0.0952 —20.6348	0.7031 —24.4690	3.0110 —22.0310
	Mean ± SD	0.3599 ± 0.3464	3.2578 ± 3.5688	8.2292 ± 6.6332	8.2756 ± 5.4223	8.3721 ± 5.1870	3.5812 ± 4.6689
o,p'-DDT	Range	<0.0001 — 0.0820	<0.0001 — 1.4750	0.0364 — 0.1710	0.0001 — 0.2440	0.0020 — 2.4200	0.0455 —10.4010
DDD	Mean ± SD	0.0125 ± 0.0173	0.1320 ± 0.3382	0.1517 ± 0.1459	0.0824 ± 0.0592	0.2229 ± 0.4151	0.1535 ± 0.0819
o,p'-DDE	Range	<0.0001 — 0.0350	<0.0001 — 0.0795	0.0100 — 0.0840	<0.0001 — 0.3120	0.0090 — 0.1350	0.0150 — 0.1818
	Mean ± SD	0.0126 ± 0.0100	0.0211 ± 0.0245	0.0457 ± 0.0265	0.0591 ± 0.0748	0.0424 ± 0.0316	0.0533 ± 0.0436
Total *p,p'*-DDT	Range	0.0460 — 1.8108	1.2939 —21.3623	1.2103 —26.3727	0.1194 —25.4047	0.8874 —36.3285	5.5692 —31.1115
	Mean ± SD	0.5198 ± 0.4234	6.2476 ± 5.4799	13.6579 ± 3.9867	12.4778 ± 7.2702	14.8069 ± 9.0557	15.3846 ± 7.1211
Total *o,p'*-DDT	Range	0.0083 — 0.0820	<0.0001 — 1.5636	0.0508 — 0.1888	0.0036 — 0.5916	0.0574 — 2.5704	0.0678 — 0.5480
	Mean ± SD	0.0265 ± 0.0200	0.1556 ± 0.3561	0.2087 ± 0.1473	0.1483 ± 0.1183	0.2701 ± 0.4363	9.2143 ± 0.1149
Total DDT	Range	0.0845 — 1.8928	1.3399 —21.4610	1.2611 —26.4717	0.1230 —25.5416	0.9822 —38.8989	5.7099 —31.3674
	Mean ± SD	0.5464 ± 0.4329	6.4032 ± 5.6787	13.9141 ±10.0004	12.6261 ± 7.2928	15.0770 ± 9.3343	15.5989 ± 1.1267
α-BHC	Range	<0.0001 — 0.0227	<0.0001 — 0.0891	0.0028 — 0.0350	0.0032 — 0.1050	0.0070 — 0.0680	0.0068 — 0.6310
	Mean ± SD	0.0103 ± 0.0100	0.0147 ± 0.0245	0.0217 ± 0.0173	0.0232 ± 0.0200	0.0207 ± 0.0141	0.0497 ± 0.1371
β-BHC	Range	<0.0001 — 0.1031	<0.0001 — 0.1284	0.0202 — 0.1480	0.0007 — 2.1050	0.0157 — 2.1420	0.0294 — 1.3300
	Mean ± SD	0.0248 ± 0.0245	0.0178 ± 0.0300	0.1880 ± 0.3150	0.2059 ± 0.4178	0.4580 ± 0.6385	0.2854 ± 0.3781
γ-BHC	Range	<0.0001 — 0.0400	<0.0001 — 0.0616	0.0010 — 0.0691	0.0010 — 1.8683	0.0010 — 0.4630	0.0010 — 0.5799
	Mean ± SD	0.0168 ± 0.0141	0.0145 ± 0.0173	0.0270 ± 0.0316	0.1947 ± 0.4017	0.0402 ± 0.0872	0.0884 ± 0.1386
Total BHC	Range	0.0146 — 0.1154	0.0019 — 0.1460	0.0414 — 0.1680	0.0473 — 2.1330	0.0671 — 2.1630	0.0888 — 1.3950
	Mean ± SD	0.0519 ± 0.0265	0.0471 ± 0.0400	0.2366 ± 0.3151	0.4238 ± 0.5620	0.5189 ± 0.6245	0.4234 ± 0.4299
Dieldrin	Range	<0.0001 — 0.0143	<0.0001 — 0.1250	0.0072 — 0.3150	0.0028 — 0.3150	0.0150 — 3.9600	0.0131 — 0.2330
	Mean ± SD	0.0033 ± 0.0040	0.0240 ± 0.0316	0.0909 ± 0.0768	0.0913 ± 0.0964	0.1242 ± 0.1162	0.0960 ± 0.0678
H. Epoxide	Range	<0.0001 — 0.0037	<0.0001 — 0.0374	<0.0001 — 0.0510	<0.0001 — 0.0520	<0.0001 — 0.0730	<0.0001 — 0.1320
	Mean ± SD	0.0005 ± 0.0011	0.0025 ± 0.0085	0.0115 ± 0.0200	0.0081 ± 0.0141	0.0147 ± 0.0179	0.0104 ± 0.0300

TABLE 4.—*Concentration (ppm) of organochlorine insecticides in adipose tissue of Israelis, male and female*

Compound		Age group and number of cases					
		Stillborns 44	0-11 mos. 40	5-24 yr. 61	25-44 yr. 53	45-69 yr. 63	70+ yr. 46
p,p'-DDT	Range	0.0028 — 1.2500	<0.0001 — 5.0000	0.2940 —23.3330	0.0042 —11.1200	0.0333 —24.0000	0.3570 —34.2850
	Mean ± SD	0.1824 ± 0.2490	2.4301 ± 2.1870	3.8953 ± 4.0577	2.9742 ± 2.7327	5.1738 ± 4.1645	5.7204 ± 6.8687
p,p'-DDD	Range	<0.0001 — 0.1125	<0.0001 — 1.7140	0.0555 — 3.0440	0.0091 — 2.1210	0.0709 —14.1025	0.1085 — 7.0450
	Mean ± SD	0.0139 ± 0.0200	0.1139 ± 0.4602	0.9323 ± 1.2463	0.7545 ± 0.6045	1.3167 ± 1.8773	1.4712 ± 1.4030
p,p'-DDE	Range	0.0175 — 1.7410	0.0860 —15.4900	0.3194 —21.2698	0.0952 —55.0310	0.7031 —24.4690	2.1110 —31.2810
	Mean ± SD	0.4621 ± 0.4759	2.6815 ± 3.0920	9.2236 ± 8.1383	9.3997 ± 8.5836	9.3382 ± 4.9996	10.0003 ± 6.0423
o,p'-DDT DDD	Range	<0.0001 — 0.0820	<0.0001 — 1.4750	0.0040 — 1.1250	0.0001 — 2.7170	0.0020 — 4.4740	0.0180 — 0.9800
	Mean ± SD	0.0111 ± 0.0141	0.0665 ± 0.2354	0.1489 ± 0.1676	0.1654 ± 0.3704	0.2962 ± 0.6262	0.2032 ± 0.1597
o,p'-DDE	Range	<0.0001 — 0.0350	<0.0001 — 0.0940	0.0100 — 0.0840	<0.0001 — 0.6110	0.0090 — 0.8850	0.0070 — 0.2080
	Mean ± SD	0.0105 ± 0.0100	0.0185 ± 0.0224	0.0542 ± 0.0557	0.0745 ± 0.1122	0.0691 ± 0.1212	0.0532 ± 0.0424
Total *p,p'*-DDT	Range	0.0460 — 3.1209	1.1288 —21.3623	0.8557 —36.6056	0.1194 —67.9005	0.8874 —43.9410	3.2243 —70.4700
	Mean ± SD	0.6832 ± 0.7036	5.7319 ± 4.9038	15.0140 ±12.2540	14.1864 ±11.5060	16.8932 ± 9.7083	18.4584 ±13.2120
Total *o,p'*-DDT	Range	0.0043 — 0.0820	<0.0001 — 1.5636	0.0508 — 1.1384	0.0036 — 2.3061	0.0486 — 4.6292	0.0369 — 1.0335
	Mean ± SD	0.0228 ± 0.0141	0.0888 ± 0.2486	0.2118 ± 0.1709	0.2484 ± 0.4020	0.3732 ± 0.6515	0.2631 ± 0.1769
Total DDT	Range	0.0671 — 3.1359	1.1184 —21.4610	0.9471 —38.7134	0.1230 —68.1350	0.9822 —48.5702	3.3065 —71.1441
	Mean ± SD	0.7060 ± 0.7038	5.8204 ± 4.9017	15.2436 ±12.3080	14.4347 ±11.5310	17.2664 ±10.1020	18.7214 ±13.2840
α-BHC	Range	<0.0001 — 0.0458	<0.0001 — 0.0891	0.0028 — 0.1240	0.0032 — 0.6200	0.0050 — 0.1800	0.0045 — 0.6310
	Mean ± SD	0.0096 ± 0.0100	0.0116 ± 0.0173	0.0267 ± 0.0224	0.0349 ± 0.0854	0.0247 ± 0.0265	0.0317 ± 0.0900
β-BHC	Range	0.0001 — 0.1150	<0.0001 — 1.0580	0.0202 — 2.0010	0.0007 — 2.9350	0.0157 — 4.7760	0.0294 — 2.9470
	Mean ± SD	0.0184 ± 0.0245	0.0477 ± 0.1640	0.2464 ± 0.4201	0.3377 ± 0.6573	0.4799 ± 0.7710	0.4353 ± 0.6715
γ-BHC	Range	<0.0001 — 0.0777	<0.0001 — 0.1090	0.0010 — 3.9170	6.0010 —12.1430	0.0010 — 0.4630	0.0010 — 1.2876
	Mean ± SD	0.0146 ± 0.0141	0.0174 ± 0.0224	0.0938 ± 0.4974	0.3270 ± 1.6631	0.0302 ± 0.0686	0.0796 ± 0.2126
Total BHC	Range	0.0054 — 0.1207	0.0019 — 1.0810	0.0414 — 4.3530	0.0232 —12.7010	0.0541 — 4.7980	0.0600 — 3.0230
	Mean ± SD	0.0426 ± 0.0283	0.0767 ± 0.1667	0.3366 ± 0.4120	0.4688 ± 0.7034	0.5347 ± 0.7616	0.5467 ± 0.6872
Dieldrin	Range	<0.0001 — 0.1180	<0.0001 — 0.1250	0.0072 — 0.3150	0.0038 — 0.4660	0.0150 — 3.9600	0.0131 — 0.8020
	Mean ± SD	0.0193 ± 0.0412	0.0209 ± 0.0283	0.1011 ± 0.1149	0.1160 ± 0.1136	0.1581 ± 0.1526	0.1353 ± 0.1360
H. Epoxide	Range	<0.0001 — 0.0102	<0.0001 — 0.0374	<0.0001 — 0.0510	<0.0001 — 0.1320	<0.0001 — 0.1180	<0.0001 — 0.1320
	Mean ± SD	0.0020 ± 0.0026	0.0015 ± 0.0059	0.0175 ± 0.0346	0.0148 ± 0.0265	0.0202 ± 0.0283	0.0103 ± 0.0173

In the 5-through-24-year age group, the mean total DDT was 15.2 ppm. DDE averaged 67.8% of total DDT-derived material. The mean total *p,p'*-DDT was 15.01 ppm and the mean total *o,p'*-DDT was 0.2 ppm. There was a significant difference in the storage of *p,p'*-DDT and total DDT between the age group under 11 months and the group 5 through 24 years ($p < 0.01$). Total BHC averaged 0.34 ppm, dieldrin 0.1 ppm, and heptachlor epoxide 0.02 ppm.

In the 25-through-44-year age group, total DDT averaged 14.4 ppm. DDE constituted 65.6% of total DDT. The mean total *p,p'*-DDT was 14.2 ppm, and *o,p'*-DDT was 0.25 ppm. Total BHC was 0.47 ppm, dieldrin was 0.12 ppm, and heptachlor epoxide was 0.01 ppm.

In the 45-through-69-year age group, the mean total DDT was 17.3 ppm. DDE constituted 60.7% of total DDT. The mean total *p,p'*-DDT was 16.9 ppm, and that of *o,p'*-DDT was 0.37 ppm. Total BHC was 0.53 ppm, dieldrin was 0.16 ppm, and heptachlor epoxide was 0.02 ppm.

In the group aged 70 years and over, the mean total DDT-derived material was 18.7 ppm. DDE constituted 59.8% of total DDT. The mean total *p,p'*-DDT was 18.5 ppm and total *o,p'*-DDT was 0.26 ppm. Total BHC was 0.5 ppm, dieldrin was 0.14 ppm, and heptachlor epoxide was 0.01 ppm.

From these data it was concluded that the storage level of DDT and derived material increased with age, the highest values being found in the 70-year-and-over age group.

Tables 3 and 4 also showed that males stored higher amounts of *p,p'*-DDT and total DDT in all the age groups, except the 0-through-11-month age group in which the DDT storage levels were of about the same order: 5.34 ppm total DDT in males and 5.82 ppm total DDT in females.

The authors found the trend for a positive age association of DDT storage in populations living in various areas of the world. In the general populations of South Africa (*25*), Thailand (*23*), Nigeria (*22*), and Brazil (*20*), the 25-through-44-year age group stored the highest amount of OCI in both sexes, after which there was a decrease in the storage level. In the general population of Uganda, the storage level of DDT was comparable in the 5-through-24- and 25-through-44-year age groups (*26*).

In the general populations of Kenya (*21*), Israel (*2*, and *this paper*), and nonwhite populations of the USA (*8*), it appears that a positive age association with DDT storage occurs in all ages.

These data suggest that the level of the OCI storage process tends to vary after the age of 45 years; in some populations it is higher and in others it is lower than in the 25-through-44-year age group. It appears reasonable, therefore, to consider the 25-through-44-year age group the best indicator of OCI storage in a community for purposes of comparison (Table 5).

TABLE 5.—*Storage of DDT-derived material in adipose tissue of 25-through-44-years-olds in several countries*

Country	Total DDT, ppm			References
	M & F	Males	Females	
Uganda	2.9	3.6	1.2	Wassermann et al. *(26)*
Kenya	4.5	4.6	4.2	Wassermann et al. *(21)*
Nigeria	6.5	7.4	5.9	Wassermann et al. *(22)*
Brazil	7.8	9.6	6.3	Wassermann et al. *(20)*
South Africa				Wassermann et al. *(25)*
White	8.5	10.5	6.6	
Bantu	6.5	8.6	4.4	
Thailand	13.0	15.5	10.2	Wassermann et al. *(23)*
Israel	14.4	15.9	12.2	This paper.

In the study carried out on OCI storage in the adipose tissue of people from Nigeria (*22*), a positive age association up to the age of 45 years was found both for DDT and dieldrin. These findings confirmed experimental data the authors had obtained in rats submitted to a high dosage of *p,p'*-DDT: namely, a parallel increase in the storage of dieldrin, although these animals had not received additional dieldrin apart from that naturally in food and water (*27*). In the present study, authors compared the concentration of dieldrin in the adipose tissue of people having: (1) high concentration of *p,p'*-DDT, and (2) low concentration of *p,p'*-DDT.

The statistical analysis revealed increased storage of dieldrin ($p < 0.01$) in the group with a higher concentration of *p,p'*-DDT.

These findings may be explained by a biochemical interrelationship of the two compounds in the animal body: the presence of a large amount of DDT interfering with the detoxication of dieldrin resulting in its accumulation in adipose tissue.

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RESIDUES IN FOOD AND FEED

Polychlorinated Biphenyl and Organochlorine Pesticide Residues in Canadian Chicken Eggs [1]

Jos Mes, D. E. Coffin, and D. Campbell

ABSTRACT

A nationwide survey in Canada of polychlorinated biphenyl (PCB) and organochlorine pesticide residues in eggs revealed an average of less than 10 ppb for both groups of compounds. PCB's and p,p'-DDE were found in all samples; at least 95 percent also contained dieldrin and p,p'-DDT. Lindane and cis- and trans-chlordane were present in 75 percent of all eggs. No significant differences were observed among the different regions of the country.

Introduction

The presence of polychlorinated biphenyls in the environment in general and the food supply in particular has been a growing concern for several years (*1,2*). A recent incident of PCB contamination (0.6-1.9 ppm) of thousands of eggs in the United States, due to contaminated poultry feed, is evidence of this environmental problem (*3*). A nationwide survey of PCB's in domestic chicken eggs in Canada was therefore undertaken; initial samples were collected in 1971.

The analytical data presented in this paper were obtained from the liquid portion of the egg only. This paper presents data on PCB's and several organochlorine pesticides.

Sampling Procedure

Twenty dozen grade A eggs were collected from each of the following five regions of the country: Eastern (Newfoundland, Prince Edward Island, Nova Scotia, and New Brunswick); Quebec; Ontario; Central (Manitoba and Saskatchewan); and Western (Alberta and British Columbia).

The stipulation was that all eggs from a specific region be produced locally. The eggs were stored at 8°C until analyzed.

Analytical Methods

All solvents were of a glass-distilled, residue-free grade, and were checked for purity. Pesticides and decachlorobiphenyl standards were 99 percent pure as verified by gas chromatography (GC). Aroclor 1260 was used as supplied by the manufacturer, Monsanto Chemical Company.

EXTRACTION

The liquid content of 12 eggs was pooled and stirred with a glass rod until thoroughly mixed. A 50-g sample was extracted for 3 min in a soil dispersion mixer (Star Manufacturing Co., St. Louis, Mo.) with a 300-ml mixture of hexane and acetone (2:1 v/v), previously warmed to 40°C. The extract was filtered through prewashed anhydrous Na_2SO_4 to remove the water, concentrated on an all-glass rotary evaporator (<25°C), and diluted up to 50 ml. A 1-ml aliquot was evaporated in a preweighed aluminum dish to determine lipid content.

CLEANUP AND SEPARATION OF PCB'S AND PESTICIDES

An aliquot equivalent to approximately 2 g of lipid was subjected to the low-temperature micro precipitation technique (*4*) and further cleaned on a 5 percent Florisil

[1] Health Protection Branch of the Department of National Health and Welfare, Tunney's Pasture, Ottawa, Canada.

column (deactivated with 5 percent distilled water after heating overnight at 140°C) (*5*). PCB's and pesticides were partly separated on a silicic acid column according to Armour and Burke (*6*), except that the silicic acid was prewashed with the eluent used for the pesticides, heated overnight at 130°C, and deactivated with 5 percent distilled water. A final cleanup for the pesticide fraction was carried out as before on a 5 percent Florisil column.

IDENTIFICATION AND QUANTIFICATION

The PCB fraction was concentrated to 1 ml; the pesticide fraction was carefully evaporated to dryness under a gentle stream of N_2 and redissolved in 1 ml of hexane. A 5-μl aliquot was injected into a Varian Aerograph Series 1400 gas chromatograph with an electron-capture detector (tritium foil) under the following conditions:

Column: ¼-in.-by-6-ft glass, packed with 6 percent OV-210 + 4 percent SE-30 on Chromosorb W(AW) 60/80 (0.6 g OV-210 + 0.4 g SE-30 + 10 g solid support)

Temperatures: Injector 220°C
Column 212°C
Detector 225°C

To give a retention time of 13 min for *p,p'*-DDT an approximate flow rate of 50 ml N_2/min was used. Standard solutions were made up to contain 50×10^{-5} μg/μl of Aroclor 1260 (5 μl were equivalent to a ½ (full-scale deflection) (FSD) for the highest peak on a 1-mV recorder) or $0.5\text{-}5.0 \times 10^{-5}$ μg/μl of pesticide, depending upon the individual response of the pesticide (e.g., 5×10^{-5} μg/μl of *p,p'*-DDT). A 10-μl aliquot of the standard pesticide solution had a ½ FSD for *p,p'*-DT.

A 5-μl injection of standard solution was made before and after every two sample injections.

PCB's and pesticides were quantitated by using peak-heights. Peaks 8, 10, and 11-15 in Aroclor 1260, according to the numbering system of Reynolds (*7*) and the Organization for Economic Cooperation and Development (O.E.C.D.) (*8*), were used for quantification.

CONFIRMATION OF PCB'S

The PCB fractions of 64 samples were pooled to give a total of approximately 13 μg of PCB.

Thin-layer chromatography (TLC) was carried out on precoated aluminum oxide (type E) F_{254} 20-by-20-cm plates (Brinkman Instruments, Ltd., Canada), activated at 110°C for 1 hr. A total of 2.5 μg of PCB from the pooled sample was spotted at a concentration of 0.25 μg/spot; two reference spots of Aroclor 1260 at 2.5 μg/spot were applied at each end of the line of origin. The TLC plate was developed in 1 percent acetone in hexane and the reference spots were made visible with $AgNO_3$ (*9*). The standard Aroclor 1260 showed two spots and the corresponding areas of the sample, as well as a blank, were scraped, eluted with hexane, and gas-chromatographed as above.

A portion of the pooled sample of PCB equivalent to 1 μg of PCB was perchlorinated and the resultant derivative was identified by GC according to Berg et al. (*10*), except that a Griffin-Worden pressure vessel (Kontes Glass Co. k-767100) was used for perchlorination.

CONFIRMATION OF PESTICIDES

All pesticide fractions, besides having been gas-chromatographed as before, were also run on a different column under the following conditions:

Column: ¼-in.-by-6-ft glass, packed with 5 percent QF-1 on Chromosorb W(AW) 60/80 (0.5 g QF-1 + 10 g solid support)

Temperatures: Injector 208°C
Column 175°C
Detector 229°C

To give a retention time of 26 min for *p,p'*-DDT, a flow rate of approximately 40 ml N_2/min was used.

The pesticide fractions of every other 10 samples were pooled and chromatographed on TLC plates as above. The five pooled samples were spotted at a concentration of 1.2-1.6 μg/spot of estimated pesticide; two reference spots of an appropriate standard pesticide mixture (2.5 μg/spot for an individual pesticide) were applied at each end of the line of origin. After development and visualization of the standards, the TLC plate was divided into five areas corresponding to the following pesticides, in order of increasing R_f value:

1. dieldrin, heptachlor epoxide, and lindane
2. cis- and trans-chlordane and *p,p'*-TDE
3. *p,p'*-DDT
4. DDMU, *o,p'*-DDT, *o,p'*-DDE, and heptachlor
5. *p,p'*-DDE, aldrin

Each area was then subdivided into six equal portions corresponding to the five pooled samples and a blank. Adsorbent from these portions was removed, and the pesticides were eluted with hexane and rechromatographed on both GC columns.

CONTROLS

Samples were spiked by adding 1 ppm and 1-10 ppb levels of Aroclor 1260 and pesticides to 50 g of whole liquid egg before extraction.

At different times during the survey three blanks were run through the complete analytical procedure, starting with a simulated extraction using the same solvent mixture used for the egg samples.

Results and Discussion

Results in Table 1 indicate that the mean level of all residues was below 10 ppb. A PCB and pesticide problem in eggs on the Canadian market apparently does not exist.

TABLE 1.—*PCB and pesticide residues in whole liquid Canadian chicken eggs*

Compound	Average ppb [1]	Maximum ppb observed	Percentage of samples containing residues
PCB's as Aroclor 1260	8	27	100
Lindane	3	10	75
Heptachlor	2	10	67
Heptachlor epoxide	1	3	70
p,p'-DDE	7	110	100
Dieldrin	1	6	96
p,p'-DDT	5	192	98
trans-Chlordane	2	8	78
cis-Chlordane	1	4	81

[1] Average derived from a total of 100 samples, each sample representing one dozen eggs.

The maximum PCB residue was close to 0.03 ppm and the maximum total DDT level (sum of *p,p'*-DDT and *p,p'*-DDE levels) was close to 0.3 ppm, although the latter represents a single instance of high *p,p'*-DDT and *p,p'*-DDE levels in the same sample. The second-highest total DDT value was 0.07 ppm.

The low levels found in this investigation ought not to be taken as absolute, since recovery studies for eight different egg samples ranged from 25 to 115 percent at the 1-10 ppb level. PCB's, *p,p'*-DDT and *p,p'*-DDE, however, had better than 50 percent recovery at all times. Egg samples spiked at the 1 ppm level had >80 percent recovery, except for lindane which had 60 percent recovery. The blanks gave no significant findings.

The low level of *p,p'*-DDT and *p,p'*-DDE in chicken eggs may reflect low pesticide intake by the chickens, since eggs are an important elimination route for these pesticides (*11*).

Table 2 shows a rather even distribution of PCB's and pesticides. The mean levels of *p,p'*-DDT in the Western and Eastern regions were relatively higher than in the rest of the country only because of one or two individual high values.

The GC patterns of PCB's in individual egg samples were similar to those of Aroclor 1260. During confirmation of PCB's on TLC plates two distinct areas were observed which, when scraped and eluted, had two GC patterns consisting of the following Aroclor 1260 peaks numbered according to the system of Reynolds (*7*) and O.E.C.D. (*8*):

	Fraction 1 ($R_{p,p'-DDE}$ 1.15)	Fraction 2 ($R_{p,p'-DDE}$ 1.00)
most of:	8,9,11,13,15,17,18	10,12,14,16
some of:	10,16	9

TABLE 2.—*Regional distribution of PCB and pesticide residues in Canadian chicken eggs*

Compound	Average ppb [1,2]				
	Western Region	Central Region	Ontario	Quebec	Eastern Region
PCB's as Aroclor 1260	7	9	10	7	8
Lindane	trace	4	5	3	1
Heptachlor	trace	3	4	3	1
Heptachlor epoxide	1	1	1	1	trace
p,p'-DDE	9	8	7	5	8
Dieldrin	2	1	1	1	1
p,p'-DDT	12	4	2	1	8
trans-Chlordane	trace	3	3	3	1
cis-Chlordane	trace	2	2	1	1

[1] Average derived from a total of 20 samples from each region; each sample represents one dozen eggs.
[2] Trace = <1 ppb.

The combination of both fractions gave a GC pattern quite similar to Aroclor 1260, except for peak 10 which was considerably higher. The latter may be an indication of the presence of Aroclor 1254, but no attempt was made to correct for it.

All pesticides reported in the tables were confirmed by TLC as described, including chlordanes whose presence in eggs has been reported earlier (*12*). Although *o,p'*-DDT was suspected in several samples and had been previously reported in eggs (*12*), this pesticide could not be confirmed by the TLC procedure. Traces (<1 ppb) of *p,p'*-TDE were also observed but not confirmed. Peaks similar in retention time to aldrin were occasionally found, but could not be confirmed on QF-1.

The average lipid content for whole liquid egg obtained with the hexane/acetone extraction was 10.93 percent ± 1.05. Data in Tables 1 and 2 may be converted to ppb on a lipid basis by using a factor of approximately 9.

Acknowledgment

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RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

DDT Plus PCB's in Blubber of Harbor Seals

Raymond E. Anas [1]

ABSTRACT

Samples of blubber from 13 harbor seals (Phoca vitulina richardii) *were collected in 1971 from San Miguel Island, Calif.; the Columbia River, Oreg.; Puget Sound, Wash.; and the Pribilof Islands, Alaska. Total amounts of DDT plus PCB's ranged from 380.7 to 2,350.0 ppm in five San Miguel Island seals; 459.4 to 1,620.0 ppm in two Puget Sound seals; 27.7 to 109.9 ppm in three Columbia River seals; and 6.8 to 27.8 ppm in three Pribilof Islands seals. There was no indication of loss of total DDT plus PCB's in three samples reanalyzed after 2 years in frozen storage.*

Introduction

Organochlorine pesticides have been found in seals in widespread parts of the world (*1,2*), but few data have been published on pesticides in harbor seals (*Phoca vitulina richardii*) from the eastern North Pacific Ocean. Up to 142 ppm DDE, 7.1 ppm DDD, and 2.6 ppm DDT were found in blubber from two harbor seals taken off central California (*3*). Up to 1,039 ppm total DDT and 145 ppm PCB's (polychlorinated biphenyls) were found in California sea lions (*Zalophus californianus californianus*) from San Miguel Island, Calif. (*4*), one of the areas sampled in this study. This report documents the amounts of DDT and its metabolites plus PCB's in the blubber of 13 harbor seals (*Phoca vitulina richardii*) collected in 1971 from four areas of the eastern North Pacific Ocean: San Miguel Island, Calif.; Columbia River, Oreg.; Puget Sound, Wash.; and the Pribilof Islands, Alaska.

Harbor seals of the subspecies *richardii* are nonmigratory and occur in the eastern North Pacific Ocean from Mexico to the Bering Sea. If food species preyed on by the seals do not migrate far, these seals might be used to locate geographical areas where organochlorine hydrocarbon levels are high. Harbor seals living off British Columbia feed near shore on demersal fish and octopus, although some migratory Pacific salmon (*Oncorhynchus* sp.) are eaten seasonally (*5*). Harbor seals feeding off Amchitka Island, Alaska, eat Atka mackerel (*Pleurogrammus monopterygius*) and octopus (*Octopus* sp) (*6*). A study of harbor seals in Washington showed that cods, flounders, Pacific herring, and sculpins made up 93 percent of the total diet. Squid and octopus make up another 6 percent (*7*). Harbor seals apparently feed on both migratory and nonmigratory species.

Sampling Procedures

All seals appeared to be in good health at the time of collection, except one seal from Washington which had apparently died from a deep laceration from a previous injury. Samples were collected by taking about 100 g of blubber from the belly area of each seal, near the midline and anterior to the mammaries. Blubber was

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selected for this study because organochlorine compounds are lipophilic (*1*). Samples were placed in new glass bottles and shipped frozen to the testing laboratory, WARF Institute, Inc., Madison, Wis.

Ages were estimated by counting layers of dentine in upper canine teeth (*8,9*). The error in assigning ages to harbor seals is not known. Body length was measured from the tip of the snout to the end of the tail, with the seal placed on its back.

Analytical Procedures

Samples of blubber were analyzed by gas-liquid chromatography for the following organochlorine compounds: aldrin, chlordane, endrin, heptachlor, heptachlor epoxide, methoxychlor, toxaphene, dieldrin, benzene hexachloride (BHC), PCB's, and the *o,p′* and *p,p′* isomers of DDE, DDD, and DDT. Only BHC, PCB's, and the *p,p′* isomers of DDE, DDD, and DDT were detected in 13 samples analyzed in 1971 and in 3 samples rerun in 1973. Confirmatory tests by use of thin-layer chromatography (*10*) on the rerun samples confirmed DDE, DDD, DDT, and PCB's, but not BHC. Levels of BHC will not be reported because of the negative confirmatory test.

To determine concentrations of the various organochlorine compounds, the entire 100-g sample of blubber was cut up while frozen, run through a hand food grinder, and transferred to ether-rinsed 4-oz glass jars. A random 5-g portion was placed in a tared 100-ml beaker and the weight was recorded. The sample was then dried at 40°C for 3 to 5 days, weighed for moisture content, and transferred to a 100-ml volumetric flask using petroleum ether. The blubber consisted almost entirely of fat and dissolved entirely in the petroleum ether. No weighable amounts of insolubles were found. Cleanup was carried out on a Florisil column following standard procedures (*11*). Next, the resulting solutions were concentrated to 10-15 ml and made to 25 ml with hexane. Sample solutions of 10 μl or less were injected into a Barber-Coleman Model 5360 gas chromatograph with an Sr90 electron-capture detector and the following instrument conditions:

Columns:	4-ft-by-4-mm glass packed with 5 percent DC-200 on gas chrom Q 80/100 (packed with 3 percent OV-17 on gas chrom Q 100/120 for BHC). An OV-17 + QF-1 column was added in 1973.
Temperature:	Detector 250°C; injector 230°C; column 210°C (180°C for BHC).
Carrier gas:	Nitrogen at a flow rate that gave *p,p′*-DDT a retention time of 6-8 minutes (12-14 minutes for BHC).

Peak heights were measured to quantitate organochlorine pesticide residues. Recovery rates ranged from 80 to 90 percent for all organochlorine compounds. Recoveries were run through the entire procedure, including the drying step. No corrections were made for recovery rates. The lower limits of detection were 0.02 ppm for DDE, DDD, and DDT, and 0.05 ppm for PCB's. Pesticide levels were only slightly higher on a fat basis than on a wet-weight basis, so results are reported here on a wet-weight basis only.

Aroclor 1254 was used as the standard for PCB's. Only the peak between DDT and DDD was used for estimation of PCB's, so the values for PCB's are not precise. Values for total DDT undoubtedly include some PCB's. To avoid possible errors, total DDT and PCB's were summed.

Because of high laboratory costs, only one sample was analyzed by the silicic acid method to separate DDT from PCB's (*12*). The blubber from this seal, a 176-cm male from San Miguel Island, had 2,110.0 ppm DDE, 3.98 ppm DDD, 96.1 ppm DDT, and 572.0 ppm PCB's. Total DDT plus PCB residues were 18 percent higher from the silicic acid method than were results from regular analyses. This difference is within the limits of analytical variability.

Glass bottles used to store the samples had paper lid-liners. The amounts of organochlorine compounds found in a sample of unused lid-liners would have had a maximum effect of 0.086 ppm total DDT plus PCB's. Because the additions, if they occurred, would have little effect on the observed values, data were not adjusted.

Results

Levels of total DDT plus PCB's ranged from 380.7 to 2,350.0 ppm (geometric mean 610.7) in San Miguel Island seals, 459.4 to 1,620.0 ppm (geometric mean 862.7) in Puget Sound seals, 27.7 to 109.9 ppm (geometric mean 62.5) in Columbia River seals, and 6.8 to 27.8 ppm (geometric mean 11.3) in Pribilof Islands seals (Table 1). Geometric means were computed because of the skewed distributions of the organochlorine compound residues. The organochlorine levels overlapped in San Miguel Island and Puget Sound seals, and in Columbia River and Pribilof Islands seals. Levels in the Columbia River and Pribilof Islands seals did not overlap the levels in San Miguel Island and Puget Sound seals. A one-way analysis-of-variance test shows that the mean total DDT plus PCB levels differed significantly between areas ($P<0.01$).

The Pribilof Islands are isolated from areas of high population and industrialization; the Columbia River drains large agricultural areas in Oregon, Washington, and Idaho; and San Miguel Island and Puget Sound are close to highly industrialized areas. Harbor seals taken near heavily populated and industrialized areas had the highest total DDT plus PCB levels, and seals

TABLE 1.—*Total DDT* [1] *plus PCB's in blubber of harbor seals collected in 1971*

Location	No. of seals	Month collected	Age, yr	Length, cm	Moisture, %	Fat, %	Total DDT + PCB's (mg/kg; wet wt)	
							Geometric mean	Individual values
California: San Miguel Island	5	June	9-30+	153-176	0.9-1.4	96.6-99.2	610.7	380.7; 418.5; 435.5; 518.3; 2,350.0
Oregon: Columbia River	3	May	2-4	112-126	1.8-3.6	96.2-100.0	62.5	27.7; 80.4; 109.9
Washington: Puget Sound	2	June-August	1	95-119	2.4-5.0	90.5-95.1	862.7	459.4; 1,620.0
Alaska: Pribilof Islands	3	August	([2])	135-175	2.6-6.2	92.8-100.0	11.3	6.8; 7.1; 27.8

[1] DDE + DDD + DDT.
[2] Canine teeth lost.

taken from the most isolated area, the Pribilof Islands, had the lowest levels. However, larger samples are needed to verify these results. Variability due to sex and age could not be evaluated for these data because of the small sample sizes. Maximum residue levels occurred in male seals in each area, but the oldest seals did not have the highest total DDT plus PCB levels within an area.

After 2 years of frozen storage, two samples from San Miguel Island and one from the Pribilof Islands were reanalyzed. Total DDT plus PCB values were +18 percent, −16 percent, and −3 percent, respectively, of the original values. Results from the original and rerun samples are within the limits of analytical variability and indicate no significant changes in residue levels.

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Chlorinated Hydrocarbon and Mercury Residues in Woodcock in the United States, 1970-71 [1]

Donald R. Clark, Jr., and M. Anne Ross McLane

ABSTRACT

During late 1970 and early 1971, 229 woodcock (Philohela minor) *were collected from 23 Eastern and Midwestern States. Analyses for chlorinated hydrocarbons and mercury in these migratory birds showed generally low levels which are not considered dangerous to human consumers. In this survey, Louisiana woodcock had lower residues of heptachlor epoxide and DDE than those tested in a 1965 survey. PCB levels, however, may have increased. Mirex levels were greatest in Mississipi and Louisiana woodcock.*

Pooling of birds and averaging of individually analyzed birds did not provide equivalent estimates of equivalent residues; pool values tended to be larger and more variable. Levels of six chlorinated hydrocarbons and mercury were negatively correlated with the latitude of the collection site. However, this relationship seemed weakest for PCB's. Among eight chemical residues, PCB levels were most often correlated with levels of the other seven. Levels of chlorinated hydrocarbons in wings were correlated with levels in breast muscle and in carcass; however, mean levels of certain residues differed significantly among wing, muscle, and carcass even when compared on a lipid basis.

Introduction

From October 1970 to February 1971, 229 woodcock (*Philohela minor*) were collected for chemical analysis from 23 Eastern and Midwestern States. Survey objectives were to establish base residue levels of organochlorine insecticides, PCB's, and mercury, and to determine the geographic distribution of mirex. Providing impetus for this survey was an earlier Canadian study (*1*) which effected the closing of the 1970 woodcock hunting season in New Brunswick, Ontario, Canada. At that time a series of 46 woodcock showed a range of 3 to 771 ppm (lipid weight) DDT plus metabolites, with a weighted mean of 60 ppm. In a more comprehensive study in 1973, woodcock averaged 25.8 ppm (lipid weight) DDT and metabolites from 164 analyses of 527 woodcock from New Brunswick (*2*).

In the 1970-71 study of U.S. woodcock, data from analyses were summarized by contaminant and compared to residue levels reported elsewhere and to U.S. Government guideline levels for meat destined for human consumption. Analytical findings were put to several other uses: pinpointing of the geographic distribution of woodcock containing mirex; comparison of data from pooled samples versus averages of individually analyzed birds; measurement of north—south geographic variation in residue levels; determination of correlations of residues of various toxicants wtih one another; and quantification of the relationships of residue levels in wing versus breast muscle, and wing versus carcass.

Analytical Methods

Among the 23 States represented in this survey were localities as far north as Maine and as far south as Florida. Several Midwestern States were sampled, including Minnesota and Missouri. Table 1 lists specific States represented and the number of woodcock collected in given counties of each State. Sampling began in October in the Northern States and ended in February in the Southern States.

[1] U.S. Dept. of Interior—Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Md. 20811.

TABLE 1.—*States and counties sampled for chlorinated hydrocarbon and mercury residues in woodcock, 1970-71*

STATE	COUNTY	BIRDS COLLECTED
Alabama	Mobile	10
Arkansas	Chicot	2
	Faulkner	2
	Grant	2
	Hempstead	4
Florida	Alachua	9
	Marion	1
Georgia	Cherokee	5
Kentucky	McCracken	1
	Ohio	4
Louisiana	Iberville	16
	Pointe Coupee	4
Maine	Hancock	3
	Penobscot	5
	Washington	2
Maryland	Calvert	2
	Dorchester	1
	Montgomery	1
	Worcester	6
Michigan	Mackinac	3
	Schoolcraft	7
Minnesota	Pine	10
Mississippi	Clay	5
	Leake	5
	Oktibbeha	5
Missouri	Callaway	4
New Hampshire	Merrimack	1
	Rockingham	1
	Strafford	3
	Unrecorded locality	5
New Jersey	Atlantic	2
	Burlington	3
	Cape May	8
	Monmouth	1
	Morris	4
	Salem	2
New York	Jefferson	8
	St. Lawrence	2
North Carolina	Johnston	6
	Pender	4
Pennsylvania	Berks	1
	Bradford	5
	Centre	1
	Erie	2
	Tioga	1
Rhode Island	Unrecorded locality	5
South Carolina	Georgetown	8
	Lancaster	2
Tennessee	Coffee	1
	Hardeman/Chester [1]	2
	Haywood	2
	Lauderdale	5
Vermont	Essex/Caledonia [1]	4
	Orleans	1
West Virginia	Mason	10
Wisconsin	Portage	6
	Sauk	4

[1] Bordering counties: collection sites overlapped.

Mercury analyses were performed on 222 of the 229 birds and all 229 were analyzed for chlorinated hydrocarbons. One hundred of these birds were analyzed in five bird pools. Two pools from both Louisiana and New Jersey were analyzed; one pool from each State having a total sample of ten or fifteen birds was analyzed. Pool birds were selected randomly from the sample from each State. Pools of livers were analyzed for mercury and pools of breast muscle were analyzed for both mercury and chlorinated hydrocarbons.

To determine organochlorine residue levels for wings, breast muscle, and carcass subsamples, 40 comparison birds were chosen randomly from the 129 woodcock remaining after pooling.

Samples of muscle from birds analyzed individually for chlorinated hydrocarbons consisted of approximately one-half (20 to 30 g) the breast muscle and excluded the skin. A 5-g sample was taken from each pooled bird. Pooled samples of breast muscle for mercury analysis consisted of 1 g of muscle from each bird. Entire livers were used for both individual and pooled samples which were analyzed for mercury.

Each wing sample included both wings, with distal segment and feathers removed. Carcass samples consisted of the remainder of the bird after samples of breast muscle, the liver, wings, skin, gastrointestinal tract, head, feet, and scaled portions of the legs had been removed. All samples were homogenized prior to analysis. Analyses were performed by WARF Institute, Inc., Madison, Wis.

Samples analyzed for chlorinated hydrocarbons were weighed, air dried with sodium sulfate for 48 hours, extracted with petroleum ether : ethyl ether (17:7) in Soxhlet apparatus for 8 hours, cleaned, and separated into two fractions by passage through a florisil column of petroleum ether : ethyl ether, 95:5, 85:15. An aliquot of the first elution was passed through a standardized silicic acid—celite column with petroleum ether, hexane, acetonitrile, and methylene chloride (*3*). Analysis was performed by electron-capture gas chromatography on a Barber-Coleman Pesticide Analyzer model 5360. The column was glass, 1208 mm by 4 mm, packed with 5 percent DC-200 80/100 mesh Gas Chrom Q. Injector temperature was 240°C; column was 200°C; and detector was 245°C. The carrier gas was nitrogen at a flow rate of 80 ml/min. Lipid weight was determined from an aliquot of the extract which was reduced to dryness on a steam bath and placed in a 40°C oven 2-4 hours before weighing.

Total mercury content was determined by cold vapor atomic absorption. Samples were digested by refluxing with sulfuric—nitric acid mixture (*4*). A mixture of hydroxylamine, stannous chloride, and sulfuric acid was added to the digest to reduce the mercury II ions to mercury metal. Samples were aerated at 3 liters/min and passed through the absorption cell.

Limits of sensitivity (wet weight, ppm) were 0.05 for mercury, 0.01 for PCB's, and 0.005 for DDE, DDD, DDT, mirex, heptachlor epoxide, and dieldrin. Re-

coveries of mercury from spiked samples ranged from 85 to 98.5 percent. Percentage recoveries for chlorinated hydrocarbons were: DDE and PCB's, 75-85; DDD and mirex, 80-90; DDT, 75-80; dieldrin, 82-94; and heptachlor epoxide, 85-90. Analytical readings were not corrected for recovery. Confirmation consisted of running duplicate analyses. For mercury, five pooled liver samples and three individual livers were duplicated; for chlorinated hydrocarbons, one pooled muscle sample, three individual muscle samples, and four carcasses were duplicated.

In addition to arithmetic means, median values are given throughout this paper because all residue data were skewed with most values toward the low end of the distribution. Statistical tests were completed after $\log_{10}(x + 1)$ transformation of the data. Trace residues were entered in the computations at the stated "less than" value, and "not detected" values were entered as zeros.

Results and Discussion

WOODCOCK AS HUMAN FOOD

Data for U.S. woodcock (Table 2) show that residue levels are generally low; average levels of DDT plus metabolites are approximately one-half those of the mean New Brunswick sample reported by Dilworth et al. (2) in 1973.

Data are presented by States (Table 3) because concern for residues in game species is centered at the State level. However, sample sizes were small and samples were not selected randomly within States. Therefore, data are statistically representative only of the area(s) actually sampled and not of the State as a whole. Because woodcock were collected during the hunting season to determine those residue levels to which hunters might be exposed, annual variation by seasons is not measured by these data.

Action Guidelines of the United States Department of Agriculture (USDA) for chlorinated hydrocarbons in meat (lipid weight) of domestic animals intended for human consumption are: DDT and metabolites, 7 ppm; PCB's, 5 ppm; dieldrin, 0.3 ppm; heptachlor epoxide, 0.3 ppm; and mirex, 0.1 ppm (John Spaulding, Ph.D., Residue Evaluation and Planning Group, USDA, *personal communication*).

Tables 2 and 3 reveal that numerous organochlorine means are apparently in excess of the USDA official limits. Because of skewed distributions, medians are both lower than and more representative of the dosage likely to be encountered in any given specimen. Nevertheless, many median values also appear to be in excess. In actuality, breast muscle of woodcock contains only 1.9 ± 0.1 percent fat (mean and 1 standard error for the 40 comparison birds), whereas hamburger, for example, may contain 28 percent fat, or 15 times the mean fat in woodcock. Therefore, in considering the safety of chlorinated hydrocarbon residues in woodcock (Tables 2, 3), it is appropriate to multiply guideline levels by approximately 15. There are no median values, including those for mirex which are discussed below, which exceed these adjusted guidelines.

The maximum level of mercury in fish muscle allowed by the Food and Drug Administration (FDA), United States Department of Health, Education, and Welfare, is 0.5 ppm (wet weight) (Spaulding, *personal communication*).

Among the State pools of breast muscle analyzed for mercury, the highest residue values, 0.31 ppm for Florida and 0.30 ppm for Alabama, were below the 0.5 ppm level. Mercury levels in breast muscle of woodcock apparently present no hazards to humans according to established tolerance levels.

LOUISIANA WOODCOCK

McLane et al. (5) report residues (lipid weight) of heptachlor epoxide, dieldrin, DDE, DDD, and DDT in 33 woodcock collected January 1965 in West Baton Rouge, Pointe Coupee, and Iberville Parishes of Louisiana. Analyses of 10 birds in the present sample afford a comparison for the same area 6 years later. To make this comparison, the authors' 1970-71 data were reanalyzed according to McLane et al. (5): trace readings were used at one-half the stated "less than" values. This results in data slightly different from Louisiana values in Table 3. The mean (1.87 ppm) and median (0.88 ppm) residues for heptachlor epoxide in 1965 were higher than the mean (0.04 ppm) and median ("not detected") levels found in 1971. The mean (1.65 ppm) and median (0.48 ppm) residues for dieldrin in 1965 do not differ greatly from the mean (0.80 ppm) and median (0.56 ppm) values found in 1971. DDE was present in 1965 at mean (17.90 ppm) and median (16.15 ppm) levels which exceed the mean (6.88 ppm) and median (3.32 ppm) residues of 1971. The 1965 measurements of DDD and DDT included trace amounts of PCB's; therefore, it is not possible to judge whether DDD and DDT have increased or decreased. It would seem that mean (3.65 ppm) and median (3.92 ppm) levels of PCB's in 1971 have increased over the trace amounts present in 1965.

GEOGRAPHIC DISTRIBUTION OF MIREX

Mirex was found in breast muscle of ten individually analyzed woodcock from five States. Only one of five birds from Maryland had a residue of 0.658 ppm (lipid weight), resulting in a State average of 0.132 ppm. Two of five woodcock from Alabama had residues of 2.18 ppm and 2.20 ppm; State average was 0.876 ppm. Two of five birds from Tennessee had residues of 0.783 ppm and 5.64 ppm; State average was 1.28 ppm. One of ten birds from Louisiana contained 26.7 ppm; State average was 2.67 ppm. Mississippi showed the highest

TABLE 2.—*Residues of chlorinated hydrocarbons and mercury in U.S. woodcock, 1970-71*

Chemical [1]	Birds sampled	Birds with no detectable residue	Birds with trace residue	Residue level, ppm	
				Wet weight	Lipid weight
DDE	129	0	5		
Mean				0.217	11.2
Median				0.036	2.47
Range				0.004-8.67	0.196-432
DDT	129	46	56		
Mean				0.010	0.573
Median				0.005	0.364
Range				0-0.220	0-14.0
DDD	129	23	38		
Mean				0.030	1.64
Median				0.009	0.647
Range				0-0.870	0-55.7
Dieldrin	129	12	63		
Mean				0.018	1.07
Median				0.005	0.381
Range				0-0.550	0-30.7
Heptachlor epoxide	129	89	22		
Mean				0.003	0.188
Median				0	0
Range				0-0.082	0-8.67
Mirex	129	119	0		
Mean				0.010	0.762
Median				0	0
Range				0-0.440	0-33.6
PCB's	129	4	23		
Mean				0.075	4.65
Median				0.060	3.65
Range				0-0.43	0-25.7
Mercury	122	0	12		
Mean				0.197	—
Median				0.145	—
Range				0.05-1.1	—

[1] Mercury analyses are of liver; other analyses are of breast muscle.

levels, with four of ten woodcock containing 4.54 ppm, 10.5 ppm, 11.5 ppm, and 33.6 ppm. State average was 6.01 ppm.

State pools showed small amounts of mirex in birds killed in Minnesota, 0.714 ppm; Wisconsin, 0.620 ppm; and South Carolina, 0.545 ppm. The only other State pool with mirex was Mississippi, which had 17.7 ppm.

Mirex levels up to 0.192 ppm appeared in one carcass sample from each of the following States: North Carolina, Kentucky, Tennessee, Florida, Georgia, West Virginia, and Vermont. A woodcock from New York showed 0.141 ppm mirex in the carcass and 1.23 in the wing.

In summary, woodcock showed heaviest mirex residues in Mississippi and Louisiana, States where mirex has been used in attempts to control the imported fire ant (*Solenopsis saevissima*).

POOLS VERSUS AVERAGES

It is important to determine how well pooled samples reflect averages of individual birds because pooling is often used to reduce the number and therefore the cost of analyses in surveys of residue levels. The present data allow statistical comparison of one series of residue readings, each reading based on five birds pooled prior to homogenization and analysis, with another series of values, each value derived by averaging residue readings from five birds analyzed individually. Pool values and averages of individuals are paired: one of each is available from several States. However, geographic variation within States has not been completely accounted for in the experimental design because the collecting localities are not completely the same for each pool-average pair. Results in Table 4 show a general similarity between pools and averages. However, the similarities are not consistent as shown by the lack of significant correlation coefficients for DDT and dieldrin. Although t-tests for paried data revealed no significant differences between pairs of logarithmic means for pools and averages, means of pools were larger in five of seven cases. If more extensive experimentation reveals that pooling increases the levels of residues which are detected, explanation and quantification will be required.

TABLE 3.—*Geographic summary of chlorinated hydrocarbon and mercury residues in individually analyzed U.S. woodcock, 1970-71*

State	N [2]	Residue levels, ppm [1]														
		DDE + DDT + DDD			Dieldrin			Heptachlor epoxide			PCB's			Mercury		
		Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Fla.	5	10.6	9.50	3.03-22.1	0.98	1.11	0.34-1.58	2.34	0.89	0-8.67	7.59	6.82	0.82-15.6	0.28	0.28	0.18-0.36
Ga.	5	6.75	4.10	2.30-16.8	0.35	0.34	0.30-0.39	0.15	0	0-0.42	6.54	6.58	5.42-7.81	0.17	0.19	0.05-0.23
Ala.	5	110	7.42	5.77-502	1.52	0.59	0.18-5.78	0.08	0	0-0.39	10.9	3.68	2.70-25.7	0.61	0.68	0.29-0.92
Miss.	10	47.2	13.1	1.41-319	0.56	0.42	0.24-1.74	0.14	0	0-0.50	4.62	3.42	0-15.6	0.17	0.16	0.08-0.27
La.	10	8.19	5.12	0.67-33.1	0.87	0.67	0.13-3.41	0.06	0	0-0.38	3.65	3.92	1.38-5.17	0.25	0.25	0.13-0.36
N.C.	5	9.01	7.15	3.23-15.8	2.48	0.70	0.52-9.62	0.24	0.27	0-0.44	3.86	3.30	2.72-5.43	0.37	0.26	0.05-0.87
S.C.	5	10.2	7.27	5.55-23.7	6.67	0.60	0.31-30.7	0.05	0	0-0.26	4.36	4.13	2.30-7.75	0.57	0.33	0.17-1.10
Tenn.	5	11.3	13.0	3.39-17.0	1.43	1.10	0.40-3.42	0.37	0.32	0.29-0.52	8.25	7.86	4.55-12.5	0.35	0.26	0.20-0.66
Ark.	5	22.0	8.81	4.21-64.5	4.61	0.67	0.42-13.5	0.22	0	0-0.79	3.03	2.58	2.07-4.13	0.17	0.19	0.06-0.26
Md.	5	3.71	3.48	2.42-5.76	0.50	0.40	0.20-1.06	0.03	0	0-0.17	5.74	5.71	3.32-8.24	0.22	0.24	0.14-0.28
W. Va.	5	1.36	1.60	0.25-2.62	0.19	0.20	0-0.30	0.05	0	0-0.24	3.41	3.13	1.78-5.67	0.08	0.07	0.05-0.14
Ky.	5	2.36	1.86	1.12-4.33	1.13	0.44	0.24-2.86	0.17	0.20	0-0.27	4.85	4.88	4.31-5.43	0.09	0.08	0.05-0.14
Mo.	4	1.95	1.97	1.37-2.50	1.86	0.34	0-6.78	0.11	0	0-0.44	3.40	2.78	1.92-6.13	0.13	0.15	0.05-0.17
R.I.	5	15.6	11.7	1.29-47.6	0.88	1.04	0-2.68	0	0	—	8.58	3.38	1.47-19.5	0.13	0.13	0.05-0.22
N.J.	10	4.14	2.82	0.64-10.2	0.33	0.27	0.20-0.60	0.03	0	0-0.28	2.20	2.12	0-4.77	0.19	0.16	0.08-0.46
Pa.	5	2.64	1.68	1.30-6.00	0.39	0.30	0.20-0.83	0.21	0	0-0.80	2.61	1.88	1.51-5.17	0.10	0.07	0.07-0.17
Maine	5	3.43	0.48	0.31-15.2	0.18	0.19	0.13-0.23	0.04	0	0-0.23	2.43	2.60	1.10-3.51	0.07	0.05	0.05-0.12
Vt.	5	1.72	0.53	0.44-5.33	0.24	0.22	0.21-0.33	0	0	—	3.64	3.47	2.91-4.63	0.08	0.08	0.05-0.12
N.H.	5	3.35	2.10	1.05-8.48	0.23	0.24	0-0.37	0.11	0	0-0.29	1.03	1.41	0-2.21	0.09	0.08	0.07-0.12
N.Y.	5	2.64	0.89	0.59-6.08	0.32	0.39	0-0.62	0	0	—	6.72	5.00	2.36-14.4	0.15	0.13	0.08-0.23
Mich.	5	1.43	1.43	0.79-1.86	0.17	0.23	0-0.31	0	0	—	2.45	2.38	1.59-3.68	0.09	0.11	0.05-0.12
Wis.	5	6.92	2.68	0.39-26.0	0.22	0.25	0-0.47	0.14	0	0-0.47	4.83	3.65	1.51-12.5	0.10	0.09	0.05-0.15
Minn.	5	1.79	1.76	0.55-3.10	0.13	0	0-0.53	0.10	0	0-0.53	5.54	3.65	1.71-12.5	0.06	0.06	0.05-0.08

[1] Data for chlorinated hydrocarbons apply to breast muscle, lipid weight; data for mercury apply to liver, wet weight.
[2] N = number of woodcock in sample. Sample sizes for mercury are one less than the number indicated in the following States: Tenn., Fla., R.I., Vt., La., N.H., and N.J.

TABLE 4.—*Comparison of pools and averages of residue data for chlorinated hydrocarbons in breast muscle, and mercury in liver, of U.S. woodcock, 1970-71*

	Residue levels, ppm [1,2]						
	DDE	DDD	DDT	Dieldrin	Heptachlor epoxide	PCB's	Mercury
Means							
Averages	0.808	0.344	0.178	0.258	0.066	0.703	0.079
Pools	0.976	0.400	0.190	0.313	0.082	0.694	0.078
Variances							
Averages	0.2255	0.0728	0.0201	0.0548	0.0129	0.0357	0.0033
Pools	0.2644	0.0902	0.0169	0.1031	0.0190	0.0630	0.0030
Correlation Coefficients [3]	0.630**	0.609**	0.303	0.436	0.764***	0.579**	0.946***

[1] Data for chlorinated hydrocarbons represent lipid weight; data for mercury represent wet weight.
[2] Residue levels in pools and arithmetic averages of individual residue levels were transformed by $\log_{10}(x + 1)$ prior to calculation of these means, variances, and correlation coefficients. Sample size is 20 for chlorinated hydrocarbons and 15 for mercury.
[3] Significance levels: ** = $0.01 \geq P > 0.001$; *** = $P \leq 0.001$.

Comparison of sample variances (Table 4) shows that in five of seven cases the pool variance is larger than the average variance. None of these differences produce a significant F value when tested. Together they suggest that pool data are less reliable. If this difference is real, it may be because each State average comes from five analyses, whereas each pool comes from a single analysis. However, the mechanism is not obvious. The authors' limited data suggest that pools and averages with a sample size of five are not equivalent and that pool values are larger and more variable.

NORTH—SOUTH VARIATION IN RESIDUES

Gross inspection of the data indicated that levels of some residues increase from north to south. To examine this relationship the authors assigned each State a value from 1 to 5 as an index of latitude: 1—Florida, Georgia, Alabama, Mississippi, Louisiana; 2—North Carolina, South Carolina, Tennessee, Arkansas; 3—Maryland, West Virginia, Kentucky, Missouri; 4—Rhode Island, New Jersey, Pennsylvania; 5—Maine, Vermont, New Hampshire, New York, Michigan, Wisconsin, Minnesota. These values were then analyzed for correlation with the 23 States' average levels of chlorinated hydrocarbons (breast muscle, lipid weight) and mercury (liver, wet weight); Table 5 shows results. Number of individual birds for each State ranged from 4 to 10 with an average of 5.6 for chlorinated hydrocarbons and 5.3 for mercury.

Table 5 confirms that residues in general are negatively correlated with latitude. Furthermore, except for PCB's, the size of the correlation coefficients is positively correlated with mean residue level (Table 2, wet weight values); hence low levels are probably responsible for the smaller coefficients of DDD through heptachlor epoxide (there were 4 States where no individual muscle samples contained heptachlor epoxide and 18 where none contained detectable mirex). However, this explanation does not account for the low PCB coefficient. Whereas the insecticides presumably increase toward the South due to greater agricultural usage, PCB's originate from various sources (*6,7*) not necessarily related to latitude, and this may explain the weaker relationship. The strong correlation of mercury with latitude (Table 5) presumably reflects greater ambient mercury in woodcock habitats with decreasing latitude. How the natural and personmade sources of mercury combine to produce such a distribution is not known.

TABLE 5.—*Correlations between average residue levels in U.S. woodcock and latitude index for 23 States of collection, 1970-71* [1]

CHEMICAL	CORRELATION COEFFICIENT [2]
Heptachlor epoxide	−0.408
PCB's	−0.431*
Mirex	−0.528*
DDT	−0.530*
Dieldrin	−0.543**
DDD	−0.550**
Mercury	−0.659***
DDE	−0.670***

[1] Ppm values transformed by $\log_{10}(x + 1)$.

[2] Significance levels: * = $0.05 \geq P > 0.01$; ** = $0.01 \geq P > 0.001$; *** = $P \leq 0.001$.

CORRELATIONS OF RESIDUES WITH ONE ANOTHER

Among the eight chemical residues, levels of PCB's are most often significantly correlated with levels of other chemicals; this is true in six of seven cases (Table 6). DDT and its metabolites are closely associated with one another and with dieldrin and PCB's. The absence of heptachlor epoxide and mirex from many samples (Table 2) probably accounts for their lack of correlation with other residues. Mercury, in spite of its relative abundance (Table 2), is significantly associated with only three other materials. However, because mercury is assimilated and stored differently than the chlorinated hydrocarbons, a lesser degree of association is expected.

WING RESIDUES AS INDICATORS

Wings of woodcock obtained annually from hunters to evaluate the species' population levels and reproductive success (*8*) are now also being used to survey geographic trends in residues of chlorinated hydrocarbons (*9*). Dilworth et al. (*4*) have shown a highly significant correlation between DDT and metabolites in wing and breast muscle. Our data (Table 7) allow quantification of both wing-to-breast-muscle and wing-to-carcass relationships. Calculation of the mean difference and its 95-percent confidence interval makes it possible to predict accurately residue levels in breast muscle or carcass from amounts found in wings for this group of 40 woodcock. Of course, how these relationships might be altered by variations in overall residue levels is not known.

Wing, muscle, and carcass samples contain different percentages of fat. For our sample these values (mean ± 1 standard error) are: wing, 14.4 ± 0.3 percent; muscle, 1.9 ± 0.1 percent; and carcass, 9.3 ± 0.6 percent. To compensate for this variable, calculations were made on lipid weight data. Nevertheless, results in Table 7 show significant differences among the three tissues for DDD, DDT, and PCB's. Therefore, quantification is needed beyond the determination of a significant correlation coefficient if wing residues are to be used to predict residue levels in other tissues.

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TABLE 6.—*Correlation matrix for residue levels of 8 chemicals in 122 individual U.S. woodcock, 1970-71*

Chemical	Residue levels, ppm [1,2]							
	Mercury	DDE	DDD	DDT	PCB's	Mirex	Heptachlor epoxide	Dieldrin
Mercury	1	0.324***	0.202*	0.116	0.250*	0.082	0.063	0.114
DDE		1	0.803***	0.644***	0.436***	0.106	0.176	0.434***
DDD			1	0.749***	0.361***	0.043	0.096	0.486***
DDT				1	0.455***	0.046	0.064	0.356***
PCB's					1	−0.127	0.209*	0.208*
Mirex						1	−0.080	0.0003
Heptachlor epoxide							1	0.180
Dieldrin								1

[1] Residue levels of chlorinated hydrocarbons (breast muscle) and mercury (liver) were transformed by $\log_{10}(x + 1)$ prior to computations. Data for chlorinated hydrocarbons represent lipid weight; data for mercury represent wet weight.
[2] Significance levels: * = $0.05 \geq P > 0.01$; *** = $P \leq 0.001$.

TABLE 7.—*Levels of residues in U.S. woodcock wings as indicators of levels in breast muscle and in carcass, 1970-71*

Chemical	Residue levels, ppm [1,2]			
	Mean	t (paired data)	Mean difference ±95% confidence interval	Correlation coefficient
DDE				
Muscle	0.584			
		1.57	0.031 ± 0.040	0.955***
Wing	0.615			
		1.10	0.025 ± 0.046	0.949***
Carcass	0.640			
DDD				
Muscle	0.210			
		4.75***	0.095 ± 0.040	0.779***
Wing	0.115			
		3.28**	0.056 ± 0.034	0.862***
Carcass	0.172			
DDT				
Muscle	0.114			
		5.44***	0.161 ± 0.060	0.492***
Wing	0.275			
		4.92***	.0.092 ± 0.038	0.839***
Carcass	0.183			
PCB's				
Muscle	0.708			
		4.24***	0.115 ± 0.055	0.737***
Wing	0.593			
		1.80	0.054 ± 0.061	0.730***
Carcass	0.538			
Dieldrin				
Muscle	0.192			
		0.342	0.007 ± 0.042	0.937***
Wing	0.199			
		1.56	0.064 ± 0.083	0.581***
Carcass	0.134			
Heptachlor epoxide				
Muscle	0.075			
		0.961	0.020 ± 0.042	0.626***
Wing	0.055			
		0.693	0.004 ± 0.013	0.932***
Carcass	0.059			

[1] Residue levels in ppm lipid weight were transformed by $\log_{10}(x + 1)$ prior to computations. Sample size is 40.
[2] Significance levels: ** = $0.01 \geq P > 0.001$; *** = $P \leq 0.001$.

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Studies on the Distribution and Flux of Pesticides in Waterways Associated with a Ricefield—Marshland Ecosystem [1]

Thomas M. Ginn [2] and Frank M. Fisher, Jr.[3]

ABSTRACT

On coastal prairie and marshland in Chambers County, Tex., authors studied the distribution and flux of chlorinated hydrocarbon pesticides in waterways associated with a ricefield—marshland ecosystem. Aldrin applied with seed rice entered the aquatic ecosystem through drainage of flooded ricefields. Chemical alteration of the pesticide was observed; dieldrin was the primary breakdown product. All insecticides were distributed unevenly, exhibiting a predilection for biotic components of the ecosystem. Residue analyses of representative species of the aquatic biota indicated significant biological accumulation and passage of these refractory compounds along the food chain. Rapid localization and concentration of pesticides in living organisms was observed. Reproductive tissues exhibited a marked affinity for the pesticides. Decline of assimilated residues in both biotic and abiotic components appeared to follow a first-order reaction curve. Contamination of the aquatic environment with toxaphene during the study period resulted in a massive kill of aquatic organisms. Neither long-term effects nor significant biological magnification of toxaphene was observed. Other chlorinated hydrocarbon pesticides of unknown origin were detected, including DDE, DDD, and DDT.

Introduction

The toxicity of chlorinated hydrocarbon pesticides to aquatic organisms has been documented by many investigators (*1-8*). Effects of the pesticidal chemicals range from acute intoxication resulting in death of the organism to more subtle sublethal effects. Though only slightly soluble in water, chlorinated hydrocarbon pesticides enter the aquatic environment dissolved in minute amounts or in greater amounts adsorbed to suspended sediment particles (*9*). Rudd (*10*) asserted that water is the primary means of residue transport from a treated area to an untreated one. The presence in surface waters of such persistent, broadly toxic compounds results in exposure of the entire aquatic biota to the residues. Furthermore, in the laboratory, fish and other aquatic organisms have shown a marked ability to accumulate pesticides from the milieu. This has been attributed primarily to two factors: direct absorption of insecticides from water, and assimilation and concentration of residues from food substances. Butler (*11*) noted the phenomenon of direct absorption of residues by oysters. Chadwick and Brocksen (*12*) reported that accumulation of pesticides by fish was dependent on the concentration of pesticides in water. However, Murphy (*13*) asserted that the method of uptake was dependent on the size of the organism. In addition, accumulation of pesticides by organisms in natural aquatic ecosystems has been documented (*14-16*). This phenomenon of accumulation of pesticides by living organisms appears to be universal (*17*) and is primarily attributable to the chemical properties of these globally dispersed refractory compounds.

The effects of accumulating pesticides are for the most part unknown. Sublethal amounts of these compounds in water have been shown to retard growth (*3*), decrease reproductive success (*18*), and alter behavior (19) in aquatic organisms. Most investigations have confined themselves, however, to laboratory studies on isolated

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components of an aquatic ecosystem or isolated characteristics of an insecticide. At present, few data exist on the distribution, localization, and impact of various pesticides in natural aquatic ecosystems. Studies of residues in a few complete ecosystems have been undertaken (*16,20*).

The marshland—estuarine environment is one for which virtually no data exist on the dynamics or short-term effects of chlorinated hydrocarbon pesticides. As the habitat for a great and diverse group of aquatic species, the estuarine environment is a vitally important biological system. The authors' purpose was to investigate the dynamics and distribution of certain chlorinated hydrocarbon pesticides in a marshland—estuarine ecosystem. The primary pesticide in the investigation was aldrin applied in cultivation of rice and introduced into the aquatic ecosystem by drainage of flooded ricefields. In addition, 11 other chlorinated hydrocarbon pesticides were monitored in the organisms of this aquatic community.

Study Area

The study area was composed of approximately 8,000 hectares of coastal prairie and marshland in Chambers County, Tex., adjacent to East Galveston Bay (Fig. 1). The marshlands were used primarily for cattle grazing and the lower prairie was used for rice cultivation. Runoff from the marsh and rice-growing areas passed through a series of personmade drainage canals and natural bayous into East Bay. Salinity of drainage waters varied from 0.5 to 19 parts per thousand (ppt) during the study. Aquatic organisms inhabiting the waters were typically euryhaline—estuarine species. The marshland primarily discussed in this paper was a 300-hectare section of the above area which had been drained; it was cultivated with rice between March and August 1971. Portions of the lowlands not involved in rice cultivation were designated as control areas.

Materials and Methods

Aquatic organisms were trapped or netted in the main drainage points of treated ricefields and stored at 4°C or quick-frozen (−20°C) until time of extraction and analysis. Small organisms (*Palaemonetes, Brevoortia,* etc.) were collected to give a sample size of 30 to 60g. The lower value represented the smallest sample in this study. Routinely, dip nets and seines were used for benthic forms. Cast and gill nets were used for fish and commercial crab traps were used effectively for crabs and some fish. Preparation for extraction was a modification of the method described by A. Wilson of the U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Fla. *(personal communication).* Samples were weighed and

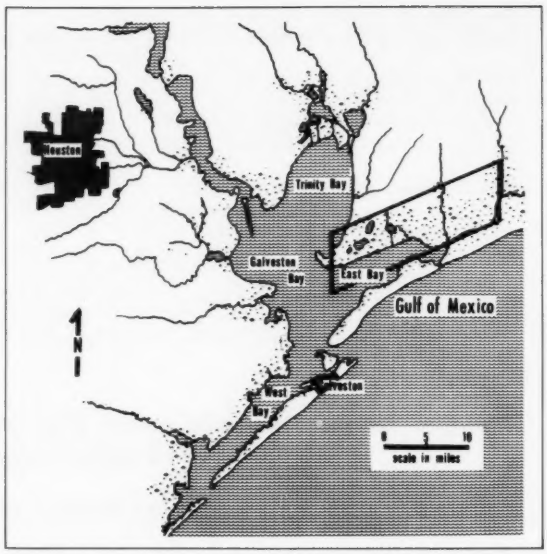

FIGURE 1.—*Location of ricefield—marshland study area on Texas coast*

placed in mason jars to which anhydrous Na_2SO_4 was added in an exact multiple of the sample weight (usually 3×). The mason jar was capped with a cutting assembly (Osterizer Corp.) and the sample was ground until a homogenous mixture was obtained. The ground sample was frozen (−20°C) for 15 to 20 minutes and reground until a free-flowing powder mixture was obtained. Repeated freezing and regrinding was often necessary to achieve such a powder. The tissue—Na_2SO_4 homogenate was either extracted immediately or wrapped in aluminum foil and stored in a freezer (−20°C). Analysis was of whole-body residues except when an organism was too large. In such cases, representative tissues were analyzed, as indicated in the text and tables.

Pesticide residues were extracted in Soxhlet extractors with petroleum ether as described by A. Wilson *(personal communication).* A weighed quantity of tissue-desiccant homogenate equivalent to approximately 30 g tissue was extracted for 4 hours with 250 ml petroleum ether (Nanograde, Mollinkrodt) at a temperature high enough to produce cycling of the solvent every 5-10 minutes (approximately 90°C). Following extraction, the petroleum ether was reduced to approximately 15 ml over steam using a three-ball Snyder column and was quantitatively transferred to a separatory funnel for partitioning over acetonitrile. The petroleum ether fraction was adjusted to 25 ml and partitioned twice over 50 ml acetonitrile. Each time the mixture was manually agitated for 1 minute and allowed to separate. The acetonitrile was collected in a crystallizing dish and evaporated just to dryness on a slide warmer (40°C).

Prolonged drying or excess heating was avoided to prevent loss of pesticides due to volatilization.

The residue was resuspended in petroleum ether and subjected to column chromatography on florisil (Floridin Co., Berkeley Springs, W. Va.). A two-fraction preparative chromatographic separation involved transfer of the residue to 10 g of florisil in a 400-by-20-mm glass chromatography column. Elution was effected with 150 ml of a 6-percent solution of anhydrous ethyl ether in petroleum ether followed by elution with 150 ml of a 15-percent solution of ethyl ether in petroleum ether. The florisil required heating at 120°-135°C for at least 5 hours before use. This procedure separated dieldrin and endrin from other chlorinated pesticide residues (*21*). The 6-percent eluate was reduced to suitable volume over steam and subjected to gas-liquid chromatography (GLC). The 15-percent eluate required further preparation. The fraction, reduced to approximately 20 ml over steam, was quantitatively transferred to a 400-by-20-mm glass chromatography tube containing 10 g of a 1:1 by weight mixture on magnesium oxide and Celite 545 (Johns Manville Co.). Elution was effected with 100 ml petroleum ether over vacuum sufficient to produce an elution rate of approximately 35 ml/min. The eluate was reduced to suitable volume, quantitatively transferred to a graduate cylinder, and adjusted to a known volume with petroleum ether prior to analysis by GLC.

Pesticide residues were recovered from core samples (2.5 by 15 cm) of soil by triple extraction with acetonitrile. Extraction was performed with wet soils to maximize recovery (*22*). A portion of each sample was retained and dried to constant weight at 130°C for determination of weight. Samples weighing 250-300 g were placed in mason jars with 100 ml acetonitrile and mixed for 2 minutes in a high-speed blender. After the soil had settled the acetonitrile was decanted into an evaporating dish through a funnel containing anhydrous Na_2SO_4 to absorb water. Extraction was repeated a second and a third time using 100 ml acetonitrile and blending for 2 minutes. Following decantation of the third extract, the soil was poured into the funnel, rinsed with acetonitrile, and discarded. The acetonitrile was evaporated to dryness on a slide warmer. The residue was resuspended in petroleum ether and subjected to a two-fraction purification on florisil. In this case, both the 6-percent and 15-percent fractions were subjected to GLC without further purification.

Dip samples of water were collected in 1-gal. brown glass bottles and refrigerated until time of extraction. All analyses were completed within 30 hours after collection. Samples of 1,500 ml were extracted in a separatory funnel by shaking for 5 minutes with 100-150 ml petroleum ether. The water and petroleum ether were allowed to separate and the petroleum ether was collected in an Erlenmeyer flask. Extraction was repeated a second and a third time. The aqueous phase was discarded and the petroleum ether phase was dehydrated with anhydrous Na_2SO_4. The extract was reduced to a known volume over steam. No further purification of this extract was necessary prior to analysis by GLC. This methodology gave 100-percent recovery of dieldrin from spiked samples. Salinity estimates were accomplished with standard hydrometers measuring density ranges from 1.000 to 1.030, or by chloride measurement using ampimetric titration with a chloridometer.

Residue analysis was by GLC with a Varian Aerograph Model 2100 dual-channel chromatograph equipped with tritium electron-capture detectors. Detection by electron capture allowed quantitation of organochlorine insecticides at amounts ranging from 10^{-11} g for lindane to 10^{-10} g for *p,p'*-DDT. Readout was on a Varian Aerograph Model 20 dual-pen recorder. On-column injection into an all-glass system was incorporated to prevent decomposition of certain organohalogen insecticides (*23*). Columns were 6-foot capillary U-tubes with 2-mm internal diameter. Packing material consisted of 80/100 mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.) solid phase with silicone oil liquid phases including 3 percent DC-200, 5 percent QF-1, a 2:1 mixture of 5 percent QF-1 and 5 percent DC-200, and a 3:1 mixture of 3 percent DC-200 and 10 percent OV-17.

Nitrogen was used as carrier gas at 65 psi and rate (soap film flowmeter) of 40 ml/min. During operation the column temperature was maintained at 190°C. The injector was maintained at 215°C. Sufficient sensitivity was achieved by operating the tritium detectors at 215°C. The detectors operated on a 90-volt direct-current mode. Though linear range of electron-capture detectors is limited (*24*), linearity was obtainable in the range of pesticide concentrations assayed (10^{-11} to 10^{-9} g).

Quantitation of data was based on comparison of printout of sample extract peak height with the peak heights of standard solutions of pesticides. Standards were injected following every third sample extract. Standard solutions were made using analytical grade chlorinated hydrocarbon pesticides obtained from the Pesticide Repository, Perrine, Fla. (See Appendix for common and systematic names of pesticides used in analysis.) Analysis was confirmed by using two liquid phases of different polarity such as DC-200 and QF-1. Another method employed binary solvent systems as described by Bowman and Beroza (*25*); this relies on solubility ratios of pesticides in immiscible solvents.

Results

Chlorinated hydrocarbon pesticides entered the aquatic ecosystem in the form of aldrin applied as a dressing on

seed rice (0.28 kg aldrin/hectare (ha.)). The cultivation process involved flooding ricefields to which aldrin-treated seed rice had been applied aerially, followed in 24-48 hours by discharging flood waters into drainage canals. Residue analyses of drainage canal water samples and ricefield soil samples taken before and after pesticide application are presented in Tables 1 and 2, respectively.

Aldrin had been applied to the field; yet dieldrin, rather than aldrin, was the primary residue detected. This was not unexpected since dieldrin, a persistent chlorinated hydrocarbon pesticide in its own right, is the epoxidation product of aldrin. The epoxidation process was doubtlessly hastened by environmental conditions of the ricefields, including elevated temperatures, high moisture levels, pH, Eh, and soil structure (*26,27*).

Concentrations of aldrin and dieldrin in water were extremely small. This is impressive, considering that these residues were the primary source of aldrin and dieldrin in most other components of the ecosystem. Based on field size (129.6 ha.) and application rate (0.28 kg/ha.), it was estimated that the total aldrin input into the field was 36.6 kg. Flood waters were 0.15 m deep, totaling 19.4 x 10^7 liters of water on the field. If aldrin were stable and completely soluble in water, a concentration of 186 parts per billion (ppb) would have been observed. Instability and insolubility of the chemical resulted in levels of 0.49 ppb aldrin and 0.58 ppb dieldrin in samples of unfiltered flood waters. Thus approximately 95 g aldrin and 113 g dieldrin were carried from the field when it was drained. It was this small amount that accounted for the residues appearing in biotic components of the aquatic ecosystem represented by the drainage canal.

TABLE 1.—*Pesticide residues detected in water from ricefield drainage canals before and after pesticide application*

TIME, WK [1]	RESIDUE, PPB (μG/L)[2]	
	ALDRIN	DIELDRIN
−2	ND	ND
−1	ND	ND
0.5	0.270	0.440
4	ND	0.172
8	ND	0.062
12	ND	0.040
13	ND	0.023
14	ND	0.034
15	ND	0.031

[1] Represents no. of weeks before (−) or after planting of aldrin-treated seed rice.
[2] ND = none detected (<μg/liter).

TABLE 2.—*Pesticide residues detected in ricefield soil before and after pesticide application*

TIME, WK [1]	RESIDUE, PPB (μG/KG)[2,3]	
	ALDRIN	DIELDRIN
−4	ND	ND
−1	ND	ND
2	—	2.8
4	10.9	10.1
5	8.3	5.1
8	ND	2.4
12	ND	2.7
15	ND	2.2

[1] Represents no. of weeks before (−) or after planting of aldrin-treated seed rice.
[2] Samples analyzed: composite core 2.5 by 15 cm, dry weight.
[3] ND = none detected (<μg/kg).

Following contamination, the decline of pesticide levels in water exhibited a pattern similar to that observed in many of the biotic and abiotic components of the ecosystem. As illustrated in Figure 2, the rate of dieldrin decline in water was proportional to the concentration of the pesticide present: i.e., a first-order reaction. Aldrin declined more rapidly, as stated earlier: after the fourth week following application, the insecticide was no longer detectable in water. The brief persistence of aldrin was no doubt the result of epoxidation of that compound to dieldrin. This characteristic of aldrin in water was important in evaluating and understanding pesticide assimilation by aquatic organisms.

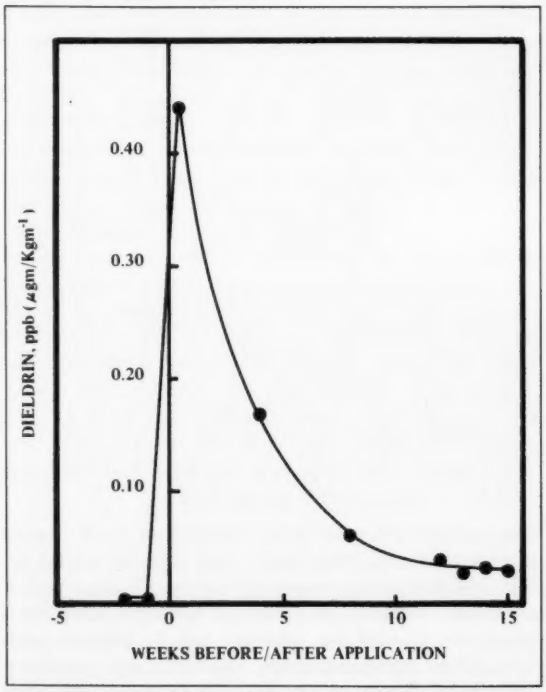

FIGURE 2.—*Dieldrin residues in ricefield drainage waters before and after planting of aldrin-treated rice*

Though 11 additional chlorinated hydrocarbon pesticides were monitored (chlordane, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, endrin heptachlor, heptachlor epoxide, lindane, methoxychlor, mirex, toxaphene), only toxaphene was detectable in water samples during the study period. Because of the chemical properties of toxaphene, it was not possible to quantitate the data obtained. Toxaphene residues and their effects on aquatic biota are mentioned later in this section. In the control marshland drainage system, water samples were taken regularly during the study period. No chlorinated hydrocarbon pesticide residues were detected.

Both aldrin and dieldrin were accumulated in soil (Table 2). The rapid decline in aldrin concentration was probably caused by loss through volatilization and decomposition; the rapid increase in dieldrin during the first 4 weeks presumably was caused by significant epoxidation of aldrin to dieldrin. The rate of loss of dieldrin from soil following the fourth week appeared to be proportional to the amount of residue in the soil. This would conform to Kearney et al. (*28*), who reported that loss of pesticides from soils followed a first-order reaction. The possibility of pesticide translocation by transported particulates has been suggested by Keith (*9*). Though the data are incomplete, pesticide analysis of bottom sediments from the ricefield drainage canal indicated localization of dieldrin of the same order of magnitude as that in ricefield soil (Table 3).

TABLE 3.—*Pesticide residues detected in drainage canal bottom sediments before and after pesticide application*

Time, wk [1]	Residue, ppb (μg/L)[2,3]	
	Aldrin	Dieldrin
−4	ND	ND
−2	ND	ND
2	ND	5.4
8	ND	2.4
15	ND	ND

[1] Represents no. of weeks before (−) or after planting of aldrin-treated seed rice.
[2] Samples analyzed: composite core 2.5 by 15 cm, dry weight.
[3] ND = none detected (<μg/kg).

Within the aquatic ecosystem represented by the ricefield—marshland drainage canals, certain representative species were chosen as monitor organisms. These species were collected and analyzed regularly throughout the study. In addition to information gathered for specific monitored organisms, pesticide data were obtained for numerous other organisms representing all levels of the food web. These organisms represented species which could not be collected regularly in the study or control areas or those which were sporadically present in the areas due to habit, life cycle, or migratory behavior.

PALAEMONETES VARIANS

Representative of plankton and nekton feeders was the grass shrimp (*Palaemonetes varians*). Chosen because of its presence in waterways of all study areas, this organism exhibited euryhaline characteristics necessary to withstand salinity changes that were likely to occur in the waterways during the study period. Before rice was planted, residue baseline in *P. varians* consisted of dieldrin in varying amounts below 20 ppb. No aldrin was detected. Within a week after water had drained from treated fields into the waterways, the dieldrin residue in the shrimp increased to 25-50 times that of baseline. A steady but much slower decline in dieldrin residues followed, as illustrated in Figure 3.

Aldrin was detected in Palaemonetes the first day of field draining and for approximately 4 weeks following. The rapid assimilation of pesticide suggested a direct absorption of residue from water. On the first day of exposure, aldrin levels were 9.4 ppb, about 40 times that of the surrounding water. Dieldrin levels were approximately the same as aldrin levels. Ten days after field drain, aldrin levels had increased to 21.7 ppb; dieldrin had increased to over 500 ppb. After 4 weeks only dieldrin was detected. At its highest level, dieldrin was accumulated to a concentration over 1,000 times the maximum concentration detected in water.

The large increase in dieldrin accompanied by only slight increase of aldrin in *P. varians* during the first week could be attributed primarily to two factors. First, aldrin concentration in the water decreased much more rapidly than did the dieldrin concentration. This difference would be mirrored in residue levels present in an organism which absorbed residues directly. Second, aldrin was probably rapidly metabolized to dieldrin by

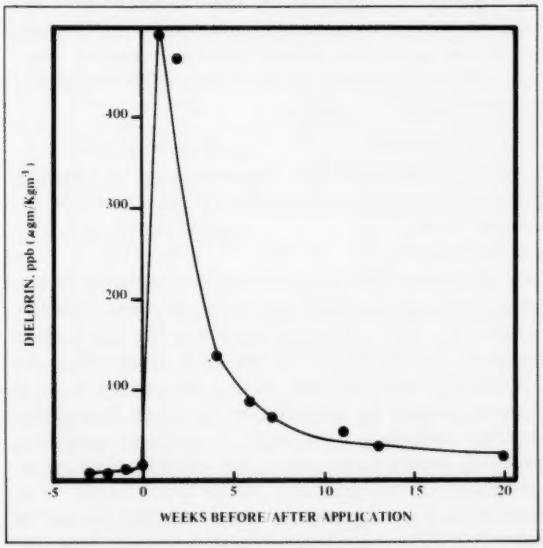

FIGURE 3.—*Dieldrin residues detected in grass shrimp* (Palaemonetes varians) *before and after planting of aldrin-treated rice*

P. varians following ingestion or absorption. Metabolic epoxidation of aldrin to dieldrin appears to be the major pathway of aldrin metabolism in living organisms (*29*).

CALLINECTES SAPIDUS

The blue crab (*Callinectes sapidus*) was chosen as a monitor organism representing a carnivorous scavenger. Dieldrin was detected in *Callinectes* collected in the aldrin-contaminated drainage system (Fig. 4). As with *Palaemonetes, Callinectes* showed dieldrin to be the primary pesticide assimilated. Levels approximately 2,000 times the water concentration were observed in this animal. Maximum concentration of 900 ppb was reached during the first week after drainage of the field. This was followed by a first-order decline over the next 20 weeks. In *C. sapidus*, aldrin was detected only during the week of field drain at a level slightly greater than 18.0 ppb.

LEPISOSTEUS OCULATUS

The spotted gar (*Lepisosteus oculatus*) represented a top carnivore of the aquatic ecosystem. Such a position within the food web should allow for biological concentration of pesticides present in the organisms of the ecosystem. The presence of aldrin, dieldrin, DDT, and its primary breakdown products was, therefore, not surprising (Table 4). Though often detected in small amounts in other environmental components, DDT, DDE, and DDD reached their highest concentration in *L. oculatus*.

It is noteworthy that aldrin was present in *Lepisosteus* much longer than in other aquatic organisms. Though usually not evident after the second to the fourth week in most food organisms of the gar, aldrin was detected for 7 weeks in *L. oculatus*. A likely explanation of this phenomenon is that, though undetectable in the food organisms, aldrin was actually present in minute quantities. The pesticide became detectable when biologically concentrated by *L. oculatus*.

Another significant discovery was the slower rate of insecticide accumulation in *Lepisosteus* after aldrin drained into the waterways. The concentration of dieldrin increased slowly over a 4- or 5-week period and then declined slowly over the next 10 weeks (Fig. 5). The slow decline in dieldrin concentration was similar to that observed in all organisms which incorporated residues: it followed a first-order reaction curve. The lag in accumulation was produced by the food chain effect. As Murphy (*13*) pointed out, a large fish primarily accumulates residues by consumption of certain food rather than by absorption from water. *L. oculatus*, therefore, probably incorporated aldrin and dieldrin through assimilation of the pesticides in its food supply. This required a multistep process which included release of pesticides into water, incorporation of residues by organisms of lower trophic levels, and ingestion of food organisms by *Lepisosteus*. The rise in pesticide levels in

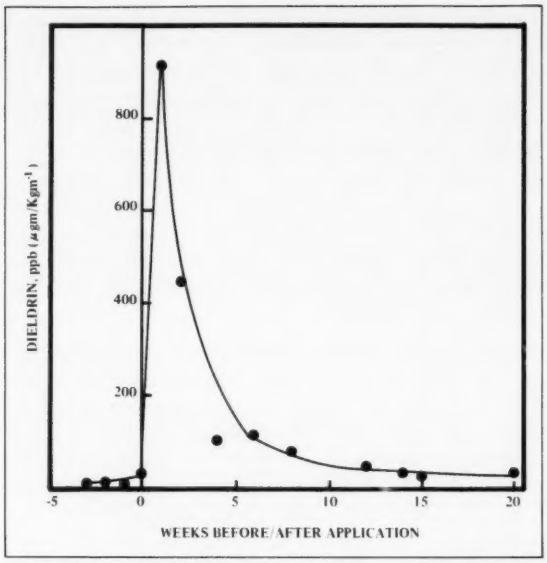

FIGURE 4.—*Dieldrin residues in blue crab* (Callinectes sapidus) *before and after planting of aldrin-treated rice*

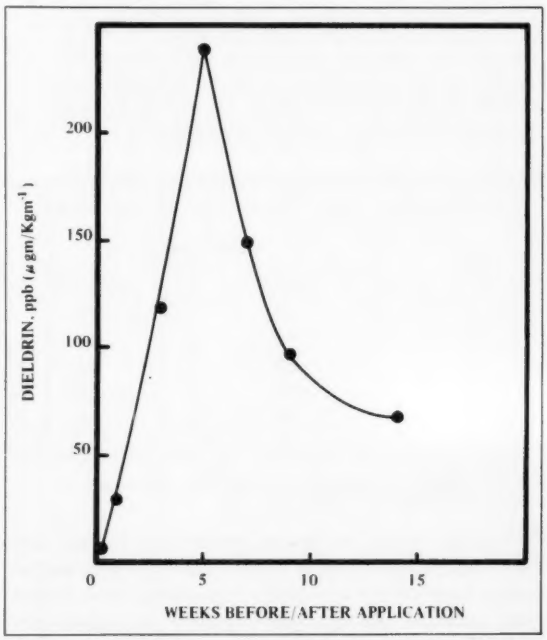

FIGURE 5.—*Dieldrin residues in spotted gar* (Lepisosteus oculatus) *before and after planting of aldrin-treated rice*

L. oculatus must by its nature lag behind the rise in residue levels in the lower trophic levels; therefore, such time shifts would be expected.

LEPOMIS MACROCHIRUS

Numerous other fish were present in the study area but were collected only on a sporadic basis. Two species,

Lepomis macrochirus (bluegill) and *Brevoortia* sp. (menhaden), were collected with some regularity. Residue data for these and the other fish collected irregularly are listed in Table 5. Residues in *L. macrochirus* showed characteristics commensurate with its position in the food chain; i.e., between the lower trophic level of *Palaemonetes* and the higher level of *Lepisosteus*. Like *Palaemonetes*, *L. macrochirus* revealed aldrin residues only while the chemical was present in the surrounding water. Dieldrin residue levels in *L. macrochirus*, however, more closely resembled those of *Lepisosteus*, reaching a peak after 4 weeks and slowly declining thereafter. DDT and metabolites were present in several *L. macrochirus*, but at levels much lower than in *Lepisosteus*.

TABLE 4.—*Chlorinated hydrocarbon pesticides detected in spotted gar* (Lepisosteus oculatus) *after pesticide application*

TIME, WK [1]	RESIDUE, PPB (μG/KG) [2,3]				
	ALDRIN	DIELDRIN	DDE	DDT	DDD
0	7.4	4.9	368.0	21.7	57.6
0	28.4	11.9	524.0	172.0	198.0
0	14.7	9.6	113.0	68.0	82.1
1	5.7	31.4	88.0	trace	29.0
3	3.9	96.8	58.0	14.2	19.3
3	7.1	153.0	107.0	34.7	79.7
5	16.3	275.0	145.0	42.2	101.0
5	12.1	198.0	82.7	5.9	14.3
7	2.6	110.0	59.0	ND	6.4
7	15.1	209.0	181.0	22.2	65.1
9	ND	98.0	191.0	ND	ND
14	ND	69.5	87.5	—	—

[1] Represents no. of weeks after planting of aldrin-treated seed rice. 0 = week of planting.
[2] Samples analyzed: 1:1 mixture of liver and muscle, wet weight.
[3] ND = none detected (<μg/kg).

BREVOORTIA

Highest recorded levels of aldrin and dieldrin in aquatic organisms were in *Brevoortia* sp. Assimilation of pesticide was rapid: extremely high levels were reached during the first day of exposure. This is understandable in view of the findings of Murphy (*13*) concerning uptake of residues by fish. In contrast to large fish, small fish assimilate residues primarily by absorption from water rather than by consumption of any foods. *Brevoortia* collected were in the 10-40-mm range and probably absorbed the pesticide directly from the water. As a result, pesticides were assimilated with exceptional efficiency and little time lag.

DIVERSE FISH

Pesticide data from the diverse fish listed in Table 5 are significant from the standpoint of survey. Dieldrin residues were observed in all fish collected after aldrin had been introduced into the habitat, and the residues persisted for a substantial length of time. These fish represent food sources of many organisms of higher trophic levels.

As mentioned previously, residues of toxaphene were detected in waters of the study area. This was the result of contamination of the drainage canals during aerial application of an unknown concentration of toxaphene to a ricefield in the study area as treatment for grasshopper infestation. The application of toxaphene was discovered following residue analysis of fish carcasses sampled from a massive fish kill which followed the spraying. The extreme sensitivity of fish to toxaphene (*5*) and the unusually high solubility of toxaphene in water (3 ppm) were factors which indicated acute toxaphene poisoning as the source of the fish kill. The number of fish killed was estimated in the tens of thousands and included catfish, menhaden, bluegill, carp, mullet, and numerous species of minnows and fry. The population of grass shrimp was so reduced after the toxaphene application that no further sampling of the organism was possible for the following 6 weeks.

Because of the nature of toxaphene, residue levels could not be quantitated. Only minute quantities of toxaphene were detected in the water following the fish kill. Furthermore, the limited persistence of toxaphene prevented accumulation of residues in most organisms of the ecosystem. Residues were detected in several aquatic feeding birds (Louisiana heron: *Hydranassa tricolor;* lesser yellow legs: *Totanus flavipes;* and black skimmer: *Rynchops nigra*), in two species of live fish (gar, and flounder: *Platichthys flesus*), and in certain aquatic insects (dytiscids). Blue crabs sampled contained no detectable residues, nor were toxaphene residues detected in any other terrestrial organisms of the area.

The significance of the input of toxaphene into the ecosystem was difficult to evaluate. Reduction of organisms was temporary and repopulation occurred within days or weeks. No long-term changes in species composition and diversity were noted. The lack of persistence of the pesticide prevented significant biological accumulation of the residue.

RESIDUES AND REPRODUCTION

The increased metabolic activity accompanying reproduction and the high lipid content of eggs would favor accumulation or deposition of pesticides in both reproductive organs and eggs. The deposition of pesticides in reproductive materials could have an important effect on the reproductive success of an organism and ultimately could affect the natural history of the species.

Levels of pesticides were determined for body tissues and eggs of two *Lepisosteus oculatus* collected in the study area and are reported in Table 6. In the case of nearly every residue present, a tenfold increase in con-

TABLE 5.—*Pesticide residues present in bluegill* (Lepomis macrochirus), *menhaden* (Brevoortia *sp.), and other species of fish before and after pesticide application*

Time, wk [1]	Organism	Residue, ppb (μg/kg)[2,3]				
		Aldrin	Dieldrin	DDE	DDT	DDD
−20	*L. Macrochirus*	ND	7.7	ND	ND	ND
−10	"	ND	34.0	12.0	ND	ND
0	"	24.5	92.0	39.8	13.3	17.4
0	"	28.6	45.0	21.6	ND	7.5
4	"	ND	410.0	30.8	ND	ND
10	"	ND	64.6	—	—	—
15	"	ND	7.3	ND	ND	ND
−20	*Brevoortia* sp.	ND	4.7	5.7	ND	ND
0	"	550.0	1380.0	25.2	ND	ND
0	"	830.0	2200.0	29.5	ND	ND
8	"	ND	81.4	ND	ND	ND
8	"	6.1	160.0	16.9	ND	ND
10	"	ND	80.0	13.8	ND	ND
11	"	ND	32.5	—	—	—
−2	*Micropogon undulatus* (croaker)	ND	2.9	33.6	ND	ND
8	*Syngnathus* sp. (pipefish)	46.8	555.0	48.6	ND	ND
12	*Mugil* sp. (mullet)	ND	104.0	15.2	ND	ND
13	"	ND	53.0	—	—	—
13	*Ictalurus punctatus* (catfish)	—	107.0	—	—	—
13	*Platichthys flesus* (flounder)	—	235.0	—	—	—

[1] Represents no. of weeks before (−) or after planting of aldrin-treated seed rice.
[2] Samples analyzed: whole body, wet weight.
[3] ND = none detected (<μg/kg).

TABLE 6.—*Pesticide residues detected in tissues and eggs of* Lepisosteus oculatus *(spotted gar) after pesticide application*

Time, wk [1]	Tissue	Residue, ppb (μg/kg)[2,3]				
		Aldrin	Dieldrin	DDE	DDD	DDT
8	Liver-muscle	2.6	110.0	59.0	ND	6.4
8	Eggs	22.3	1210.0	715.0	54.6	131.0
8	Liver-muscle	15.1	209.0	181.0	22.0	65.1
8	Eggs	49.8	1280.0	1220.0	148.0	290.0

[1] Represents no. of weeks after planting of aldrin-treated rice.
[2] Samples analyzed: tissue, wet weight.
[3] ND = none detected (<μg/kg).

centration was seen in eggs over the concentration in other body tissues. Such a phenomenon might be the result of high lipid content of eggs or possibly an active excretion of pesticides into reproductive materials. Both the dieldrin level and the high content of DDE could critically affect reproductive success of these fish. Environmental levels of dieldrin producing 50 percent mortality in various fish were reported by Moore (*30*) to range from 100 to 250 ppb. The level of dieldrin in the microenvironment of the embryos in these fish eggs exceeded those levels.

Discussion

Our data suggest that the introduction of an organochlorine insecticide such as aldrin into a natural ecosystem is followed by certain predictable physical and chemical events. In the case of aldrin, specifically, one of the first events is a chemical alteration of the insecticide structure. In the present study epoxidation of aldrin to dieldrin occurred in both biotic and abiotic components of the ecosystem. The extent of the conversion of aldrin to dieldrin was evidenced by the fact that the primary, and often only, residue detected in certain components of the ecosystem was dieldrin. Though detected in certain components early in the study, aldrin was noticeably absent in all components later on.

Insecticide residues within the ecosystem became unevenly distributed, exhibiting a predilection for biotic components. The phenomenon of biological magnification was evident at all trophic levels. Concentrations of residues in organisms ranged from 500 to 10,000 times the maximum residue concentration in water. Evidence of accumulation and passage of pesticides along food chains exists in the temporal relationships of biocide assimilation. Biological magnification and passage of insecticides along the food chain requires a stepwise process whereby residues are assimilated in lower trophic levels and subsequently passed to higher and higher levels. Such a process would exhibit a chronological sequence in which residues first appear in the lower trophic levels. Only after a delay allowing for intermediate links in the chain would the residues become available for assimilation by organisms of the higher trophic levels.

This phenomenon was evident in the organisms of the study area as illustrated in Figures 1-4. The higher the trophic level represented, the greater was the time lag before maximum residue concentration was reached. Delays occurred at each of several levels prior to assimilation of residue in the higher trophic levels. The assimilation and concentration of residue followed an orderly pattern which resembled closely that of energy flow through food chain relationships.

In all cases, the maximum accumulation occurred within a short time following introduction of the insecticide into the ecosystem. Peak levels were reached in all monitored organisms within days or weeks. The dissipation or degradation of residue following biological concentration exhibited certain common characteristics in almost every component of the environment. Kinetics

of insecticide loss resembled those of radioactive decay in that they were concentration-dependent. Loss of pesticide from water and most organisms appeared to follow a first-order reaction curve in which the rate of loss was directly proportional to the concentration of residue present. Mathematically this can be described as:

$$-\frac{dP}{dT} = PK$$

in which K = constant, P = residue concentration, and $-\frac{dP}{dT}$ = negative rate of change of P per unit time. A similar phenomenon was noted for residues in soil by Kearney et al. (*28*). This pattern of decline is evident in the graphic representation of residues in components of the ecosystem as illustrated in Figures 1-4. It would appear that concentration-dependent rate of decay or loss of insecticide occurred in both biotic and abiotic components of the aquatic ecosystem.

Summary

The chlorinated hydrocarbon insecticide aldrin applied to the rather discrete confines of a ricefield exhibited a dynamic behavior which resulted in dispersion of residues throughout the entire associated aquatic ecosystem. Though chemical alteration (aldrin → dieldrin) was extensive, dieldrin persisted in an active state for several months. Pesticides were rapidly concentrated in living organisms, apparently by both direct absorption of residues from the milieu and indirect assimilation of residues through the food web. Pesticides showed a tendency to concentrate selectively in certain tissues, notably reproductive tissues. This would indicate the need for careful study and evaluation of effects of such chemicals on fecundity and reproductive success of all organisms inhabiting areas of pesticide use.

Accumulation of aldrin and dieldrin in both biotic and abiotic components of the ecosystem was followed by a decline, the kinetics of which resembled a first-order reaction. This resulted in a rapid depletion or loss of extremely high levels of pesticide concentrated by organisms, but allowed for long-term persistence of low levels of chemically active compounds. Studies of long-term effects of small, sublethal doses of pesticide would seem to be the most logical method for evaluating the ultimate effect of chlorinated hydrocarbon pesticides on living systems.

Acknowledgments

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Selected Chlorinated Hydrocarbons in Bottom Material from Streams Tributary to San Francisco Bay[1]

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ABSTRACT

As part of a study of the environmental quality of San Francisco Bay, bottom material from 26 streams tributary to the Bay were analyzed for chlordane, DDD, DDE, DDT, and PCB residues. These compounds were present in essentially all streams tested. Chlordane proved to be ubiquitous, with a concentration range similar to that of the other compounds. Noteworthy was the occurrence in one stream of polychlorinated naphthalene residues. Compounds occurring in concentrations above 20 μg/kg were identified in most instances by combined gas chromatography/mass spectrometry.

Introduction

San Francisco Bay is one of the principal aquatic resources of the State of California. Millions of people residing on or near the shores of the Bay utilize its waters for recreation, waste disposal, mining of commercially valuable salts and minerals, marine navigation, fishing, and general aesthetic enjoyment. The Bay receives waters from many sources, large and small, which drain a densely urbanized area developed agriculturally and industrially. In order to assess the potential contamination of the Bay from chlorinated hydrocarbon compounds, the U.S. Department of the Interior—Geological Survey initiated a study in February 1972 to obtain background information. Of special interest was the input attributable to the numerous streams that discharge into San Francisco Bay.

The worldwide distribution of residues of DDT and PCB's (polychlorinated biphenyls) is well established (*1-11*). Furthermore it has been demonstrated (*12*) that PCB's are accumulated and concentrated in certain organisms by as much as five orders of magnitude greater than the organism's aquatic environment. Woodwell and coworkers (*13*) have studied the biological concentration of DDT residues in organisms of increasing trophic levels and found it to be more than three orders of magnitude. Most chlorinated hydrocarbon pesticides are only slightly soluble in water and usually enter the hydrologic environment sorbed onto particulate matter (*14*). Even when dispersed in aqueous media they sorb onto sediment (*15*). For example, Bevenue et al. (*16*) found that the ratio of chlorinated pesticides associated with sediment to that in solution was 9,000 to 1. PCB's, because of their similarity in chemical properties to some insecticides, can be expected to behave in a water/sediment mixture in a like manner. Depending upon flow conditions sorbed compounds may be either in transport or deposited as part of the streambed matrix. Hence it was decided to survey the numerous Bay-area streams by analyzing the bottom material.

Nisbet and Sarofim (*17*) have estimated that of the 5×10^5 tons of PCB's produced in the United States during 1930-70, approximately 3.9×10^5 tons, or 78 percent, have been lost to the environment. Riseborough and de Lappe (*18*), in discussing the mass balance of pollutants, state that in the last 15 years, 6×10^{10} g (6.6×10^4 tons) per year of DDT have been manufactured in the United States for both domestic use and export. Because DDT is intentionally applied to the earth, vir-

[1] U.S. Department of the Interior—Geological Survey, Menlo Park, Calif.

tually all of it has been lost to the environment, whereas PCB's are leaked to the environment by usage. In view of these large production figures the chlorinated hydrocarbon compounds DDT and PCB were considered the most likely pollutants to be found. Therefore, these compounds were chosen for study and this report summarizes the findings.

Sampling and Analytical Procedure

In February and March 1972, bottom material from 26 streams that discharge into San Francisco Bay was collected for analysis. Sampling sites are shown in Figure 1. The bottom material was collected in wide-mouth glass bottles equipped with Teflon-lined screw-top closures. Samples of soft bottom material were obtained by scooping directly with the glass bottle. When the texture of the bottom material prevented this method, samples were obtained by scooping the material with a chromium-plated garden trowel and then placing the sample in a bottle. All implements and bottles had been freed of any contaminants that would interfere with analysis.

Upon receipt in the laboratory the samples were wet-sieved through a screen with a 2-mm-pore diameter to isolate the material most likely to be transported during high flow. The immersed-screen method described by Guy (*19*) was followed, using water collected with the bed material instead of distilled water. The liquid phase was separated from the solid material that passed through the screen by allowing the mixture to stand for several hours and decanting as much water as possible. Finally, the sieved sample was stored at 4°C to inhibit microbial degradation until extraction was initiated.

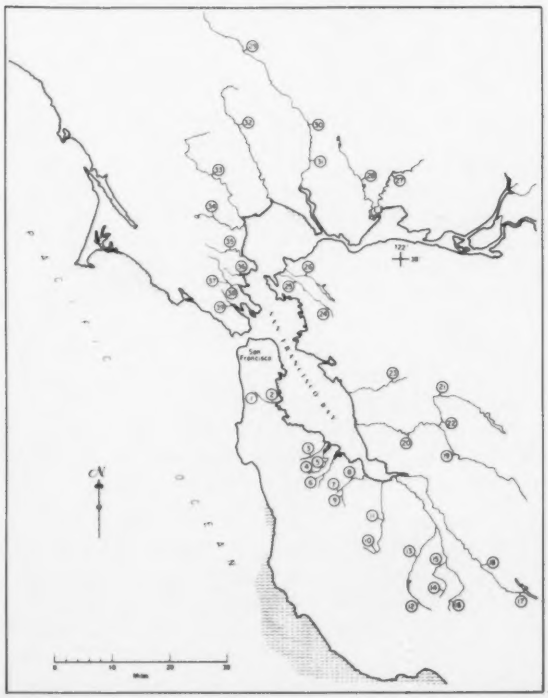

FIGURE 1.—*Map of San Francisco Bay area showing stream bed sites sampled for chlorinated hydrocarbons*

The procedure used in this study was a slightly modified version of the Geological Survey method for analyzing aquatic sediments for insecticides (*20*). A 25-g subsample of the wet bottom material was placed in a glass-stoppered flask and shaken for 20 minutes with 20-ml redistilled pesticide-grade acetone. Fifty ml of redistilled pesticide-grade hexane was added to the acetone/bottom-material slurry, and shaken for an additional 5-10 minutes. The extractant was decanted into a separatory funnel containing 500 ml of organic free distilled water. In a similar manner the extraction was repeated two more times, the only difference being that the volume of acetone used was reduced from 20 ml to 10 ml. The combined extracts were washed with three 500-ml volumes of distilled water and placed over anhydrous sodium sulfate to dry. After drying, the solvent volume was reduced to a few milliliters using a Kuderna-Danish evaporative concentrator and finally to a few tenths of a milliliter by evaporation under a stream of warm dry nitrogen. Mean recovery for the insecticides DDD, DDE, and DDT was 92, 99, and 99 percent, respectively. Mean recovery for PCB was 100 percent.

The compounds were initially isolated from interfering coextractives by adsorption chromatography. The concentrated extract was placed on a 1-by-8-cm column of alumina deactivated 9 percent by weight with water and eluted with 20 ml of hexane. The eluate volume was reduced to a few tenths of a milliliter and transferred to a second 1-by-8-cm chromatographic column of silica gel deactivated 3 percent by weight with water. Sufficient hexane was added to the column to total exactly 25 ml of eluate. At this point the elution solvent was changed to benzene and an additional 10 ml of eluate was collected separately. The PCB's were thereby isolated in the first fraction collected from the silica-gel column, and the DDT family and chlordane were in the second fraction.

Finally the extracts were treated with metallic mercury, following the procedure of Goerlitz and Law (*21*), for the removal of sulfur interferences. The purified extracts were subjected to gas-chromatographic analysis for identification and quantitation of components. Two Varian Aerograph Model 600D gas chromatographs were used. The instruments were fitted with concentric-type tritium foil electron-capture detectors and connected to Honeywell recorders through an Infotronics Model CRS 100 electronic integrator. One chromatograph was equipped with a glass column 1.8 m long by 1.8 mm i.d., packed with 60-80-mesh Chromosorb W-HP coated with OV-1 3 percent by weight. The other chromatograph was

equipped with a glass column of the same dimensions, packed with 100-120-mesh Supelcoport coated with OV-17 1.5 percent by weight and QF-1 1.95 percent by weight. Operating conditions for both chromatographs were: oven temperature, 195°C; inlet temperature, 210° C; and nitrogen carrier gas flow rate, 30 ml/min.

The DDT family of pesticides were measured by comparing the area under the chromatographic peaks with the area of appropriate standards. Multiple peak residues such as chlordane and PCB's were quantitatively measured by comparing the sum of the areas of the two most prominent peaks with the appropriate analytical standards. The approximate minimum detectable concentrations of the compounds reported, employing the stated analytical conditions, are as follows: DDE: 0.05×10^{-6} μg/kg; chlordane, DDD, *o,p'*-DDT, and *p,p'*-DDT: 0.1×10^{-6} μg/kg; and PCB: 1×10^{-6} μg/kg. When the concentration of any component of interest was great enough, combined gas chromatography/mass spectrometry was used to confirm the identity of the compound. A Finnigan Instrument Corporation Model 1015 quadrupole mass spectrometer and Systems Industries System 150 computerized controller and data output system were used. The mass spectrometer was connected to a Varian Aerograph Model 1700 gas chromatograph via a glass jet separator. The gas chromatograph was equipped with a glass column 1.2 m by 2 mm i.d., packed with Gas Chrom Q coated with OV-1 3 percent by weight. The temperature of the column oven was programmed at a rate of 6°C/min from 175° to 235°C and then held isothermally until completion of analysis. Helium was used as a carrier gas at a flow rate of 30 ml/min.

Results and Discussion

Results of the analysis of bottom material samples from San Francisco Bay Area streams are given in Table 1. Residue concentrations, uncorrected for percent recovery, are expressed as μg/kg based on the oven-dried weight of the bottom material, determined on a separate subsample. Site numbers referred to in Table 1 are the numbered sampling sites on the map in Figure 1. All residue identities in Table 1 were established by elution order when the sample extracts were subjected to column chromatography and by peak matching when analyzed by electron-capture gas chromatography. When concentration values were great enough, a gas chromatograph/mass spectrometer (GC/MS) was used to confirm the identity of a residue. Those residues that were confirmed by GC/MS are noted in Table 1.

Because this study was exploratory in design and scope, quantitative conclusions should not be drawn; however, certain generalizations can be observed. Chlorinated hydrocarbon residues were found in all stream bed samples analyzed, thus illustrating their widespread distribution in the San Francisco Bay area. Initially only the presence of the DDT family of compounds and the PCB's had been anticipated with any certainty, due to their established ubiquity. This investigation soon showed the insecticide chlordane to be as prevalent as the other target compounds; it appeared in 92 percent of the 39

TABLE 1.—*Chlorinated hydrocarbon residues found in San Francisco Bay Area streams*

Site No.	Stream	Residue concentration in μg/kg [1,2]					
		Chlordane	DDD	DDE	*o,p'*-DDT	*p,p'*-DDT	PCB
1	Colma Creek	39	3.0	2.0	1.7	3.2	3.9
2	Colma Creek	19	4.1	1.8	0.80	5.3	12
3	Belmont Creek	660	41	17	89	200	52
4	Cordilleros Creek	20	3.8	3.5	4.0	13	14
5	Cordilleros Creek	33	17	6.1	5.2	39	6.0
6	Redwood Creek	40	8.4	5.2	4.2	24	25
7	San Francisquito Creek	7.1	2.2	2.1	0.96	7.1	1.2
8	San Francisquito Creek	670	160	43	20	150	430
9	Los Trancos Creek	21	10	5.5	1.3	5.6	21
10	Stevens Creek	7.8	0.0	0.0	0.0	12.3	180
11	Stevens Creek	190	19	22	11	33	30
12	Los Gatos Creek	0.0	1.8	0.87	0.28	2.2	0.0
13	Los Gatos Creek	280	33	25	11	32	170
14	Guadalupe River	17	3.5	2.3	0.87	3.2	[3]
15	Guadalupe River	9.6	3.1	1.8	0.52	1.6	2.7
16	Alamitos Creek	46	18	26	2.2	14	610
17	Coyote Creek	83	86	40	5.7	31	14
18	Coyote Creek	71	17	11	2.6	4.3	12
19	Alameda Creek	13	5.6	4.5	0.16	0.57	11
20	Alameda Creek	52	12	9.2	2.4	2.1	30
21	Arroyo de la Laguna	200	27	23	4.5	7.3	160
22	Arroyo de la Laguna	22	3.5	4.3	0.77	1.3	33
23	San Lorenzo Creek	15	7.0	7.5	0.0	1.7	25
24	Wildcat Creek	87	18	4.1	2.7	6.5	21
25	Wildcat Creek	45	8.8	6.1	1.8	9.7	43
26	San Pablo Creek	65	2.0	3.4	2.0	3.6	27
27	Union Creek	200	45	16	2.6	2.8	140
28	Green Valley Creek	0.0	1.9	2.3	0.36	0.86	5.3
29	Napa River	10	2.2	2.7	0.78	2.3	8.8
30	Napa River	0.0	46	0.0	9.7	73	1400
31	Napa River	97	16	11	2.8	8.4	7.6
32	Sanoma Creek	4.3	0.98	1.0	0.25	1.9	5.0
33	Petaluma River	130	8.3	5.5	3.8	2.7	27
34	Navato Creek	62	3.5	3.6	2.4	10	10
35	Miller Creek	310	16	11	13	8.4	35
36	San Rafael Creek	800	120	61	38	51	350
37	Corte Madera Creek	140	12	11	8.7	48	81
38	Corte Madera Creek	66	42	42	6.7	41	11
39	Arroyo Corte Madera del Persidio	140	34	7.6	11	16	24

[1] Based on oven-dry weight of stream bed material uncorrected for percent recovery.
[2] Underlined values indicate mass spectrometric confirmation of residue identity.
[3] The presence of 55 μg/kg of polychlorinated naphthalenes (PCN) obscured any PCB's present. PCN was identified by gas chromatograph/mass spectrometer.

samples analyzed. Both the PCB's and chlordane evidenced a wide range in concentration, from 1 to greater than 1,000 μg/kg. In spite of this extreme range there does not seem to be a significant difference between the average residue concentrations of streams discharging into the Bay south of San Francisco and those discharging into the Bay north of San Francisco. Significantly, only 3 of the 39 streams tested contained residues of the DDT family in quantities greater than those of PCB or chlordane.

Although a widespread occurrence of PCB's had been expected, two sampling sites showed residue levels much higher than anticipated. On Stevens Creek site 10 had residue levels of 180 μg/kg; on Alamitos Creek site 16 had levels of 610 μg/kg. Neither area has any apparent industrial or commercial development. The sample from site 14 on the Guadalupe River contained 55 μg/kg of polychlorinated naphthalenes. This is an industrial compound similar in properties and uses to PCB's. This sample, too, came from an area of no apparent industrial activity. Nonachlor, a component of commercial-quality chlordane, was often found in relatively higher concentrations in bottom material samples than in freshly prepared standards. This suggests that nonachlor may be more resistant to degradation than either the α or γ isomers of chlordane.

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Organochlorine Residues in Golden Eagles, United States—March 1964-July 1971 [1]

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ABSTRACT

Since 1964, golden eagles (Aquila chrysaetos) *collected dead, dying, or incapacitated in the United States have been analyzed for organochlorine residues under the National Pesticides Monitoring Program. This paper reports residues found in 134 brain, heart, kidney, liver, and muscle (BHKLM), 73 brain, and 102 fat samples representing 169 golden eagles from 22 States. DDE was found most frequently, and usually in the greatest concentrations, in each sample type. Dieldrin was also common. Heptachlor epoxide, polychlorinated biphenyls (PCB's), DDD, DDT, endrin, DDMU (1-chloro-2, 2-bis [p-chlorophenyl] ethylene), and aldrin were found less frequently. Variability of residue levels was great for all types of samples, and no regional patterns were evident. The data suggest that exposure to organchlorine pesticides has remained relatively constant from 1965 through 1970. Exposure is apparently through diet and not sufficient to warrant concern for direct nonsynergistic acute toxic effects. Sublethal effects are not precluded, however.*

Of the 188 eagles found, 63 were examined for immediate cause of mortality. The most frequent causes appeared to be shooting and contact with power lines.

Of the 169 eagles found, necropsy or residue analysis suggested deaths by unnatural causes for 63. Of these, deaths due to shooting or contact with power lines were most common; other factors included trapping, collision with vehicles, and chemical poisoning. The remaining 106 birds died from undetermined causes which undoubtedly included a high proportion of natural deaths.

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Introduction

The golden eagle (*Aquila chrysaetos*), a carnivore that is terminal in its food chain, has been selected by the Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior, as one of the species to be surveyed under the National Pesticides Monitoring Program (*1*). Under this program, golden eagles found in the United States that are dead, dying, or severely incapacitated are sent through U.S. Game Management Agents or National Wildlife Refuge Managers to Wildlife Research Centers for gross examination or necropsy and for organochlorine residue analysis. This paper reports necropsy and residue findings for 169 golden eagles received in this manner from 22 States by the Denver Wildlife Research Center between March 1964 and February 1970.

Materials and Methods

Whole birds and tissue samples were usually received frozen at the laboratory and were stored frozen until analysis. Most whole birds were examined for gross evidence of death due to unnatural causes, including shooting, impact on power lines, and chemical poisoning. A few were decomposed extensively, precluding necropsy. The birds were then dewinged, debeaked, and skinned for tissue sample removal. Usually a composite of 5 g each of brain, heart, kidney, liver, and muscle (BHKLM) was selected. In some cases fat and/or brain samples were taken.

Samples to be analyzed were blend-desiccated with sodium sulphate (5 parts to 1 part of sample) and Soxhlet-extracted for 6 hours with 1:9 diethyl-ether:petroleum-

ether. Extractants were evaporated, crude lipids were determined gravimetrically, and the sample was taken up in hexane and partitioned with acetonitrile by a procedure similar to that of Mills (*2*). Additional interfering substances were removed by liquid adsorption chromatography (*2*), co-sweep distillation (*3*), or both. Various other cleanup and separative techniques, such as treatment with fuming sulfuric acid or chromatography with magnesia or alumina columns, were used when necessary. Recovery of organochlorine insecticides from eagle tissue by the above technique was 90 ± 10%.

Sample residues were usually determined by gas-liquid chromatography (GLC) using tritium-activated, concentric-tube, electron-capture detectors. The most frequently used GLC operating parameters are presented in Table 1. In most cases, a Dow-200 column was used for separation, identification, and quantitation, and a QF-1 (FS-1265) column was used for confirmation. When residue levels permitted, futher confirmation was performed by thin-layer chromatography with laboratory-prepared plates or Eastman Chromatogram (F) alumina- or silica-gel films.

TABLE 1.—*Normal operating parameters for gas-liquid chromatography, with tritium electron-capture detection*

	Columns (Glass, 5' x ⅛" O.D.)	
	QF-1	Dow-200
Liquid phase	5%	5%
Mesh size, H.P. chromasorb W	80-100	80-100
N_2 flow rate, ml/min	50	50
Temperature, °C	175	185
Retention time of aldrin, min	3.3	6.5

Plates and films were developed with 0.5% methanol and 2.5% benzene in 2,2,4-trimethyl pentane (v/v); spots were visualized by silver nitrate spraying and ultraviolet exposure. The sensitivity of this procedure was greater than 0.1 ppm for organochlorine residues and 1.0 ppm for PCB's.

Results and Discussion

Median values are presented instead of means because of the skewness of the data and the need for comparison with the literature. Residue levels reported in this study have not been corrected for percent recovery of pesticides from eagle tissues.

Organochlorine residues were determined in 134 BHKLM, 102 fat, and 73 brain samples representing 169 golden eagles. Table 2 shows the frequency of occurrence of the nine organochlorine compounds found in the samples, DDE being most ubiquitous. As shown in Tables 3-4, residues were generally greatest in the fat samples, intermediate in BHKLM samples, and least in the brain samples.

TABLE 2.—*Frequency of occurrence of nine organochlorine residues in golden eagle samples*

	Number of samples containing residues [1]					
	BHKLM (134)		Fat (102)		Brain (73)	
Organochlorine Residues	No. with residues	% of total	No. with residues	% of total	No. with residues	% of total
DDE	127	94.8	100	98.0	65	89.0
Dieldrin	68	50.7	69	67.6	19	26.0
Heptachlor epoxide	23	17.2	32	31.4	7	9.6
PCB's (polychlorinated biphenyls)	20	14.9	13	12.7	15	20.5
DDD	10	7.5	13	12.7	1	1.4
DDT	3	2.2	8	7.8	3	4.1
Endrin	2	1.5	5	4.9	0	0.0
DDMU	2	1.5	0	0.0	0	0.0
Aldrin	1	0.7	3	2.9	1	1.4

[1] Total number of golden eagles represented = 169.

Table 3 gives the median, range, and number of samples with each organochlorine insecticide residue for each sample type according to State. These data are generally too variable and sample size is too small to permit meaningful statistical comparisons between States or regions. From the data available, it appears that exposure of individual golden eagles to organochlorine insecticides is quite variable but falls within approximately the same range across the country.

As shown in Table 3, DDE was found in at least one golden eagle from each of the 22 collection States. Few of the samples contained alarmingly high residues, however. Four of the fat samples contained over 40 ppm DDE (50-84 ppm), two of the BHKLM samples contained over 20 ppm DDE (24-25 ppm), and four of the brain samples contained over 2 ppm DDE (2.3-9.9 ppm). According to the general guidelines given by Stickel et al. (*4*) in their study of DDE residues in cowbirds (*Molothrus ater*), these residues are not sufficiently high to cause direct, nonsynergistic acute toxicity to eagles.

Golden eagles from all 22 States contained a median of 0.3 ppm DDE in BHKLM, 3.0 ppm in fat, and 0.1 ppm in the brain for the years 1964-70. These are higher levels than those of DDT and DDD in all sample types, indicating that most exposure to DDT and metabolites was not recent or was dietary. The DDE levels, however, are considerably lower than the lowest median DDE residue of 4.92 ppm in the carcass and 0.92 ppm in the brain of bald eagles (*Haliaeetus leucocephalus*) as reported by Mulhern et al. (*5*) for the years 1966-68, and 7.80 ppm in the carcass and 1.00 ppm in the brain of bald eagles as reported by Reichel et al. (*6*) for the years 1964-65. The lower residue content in the golden eagle compared to that in the bald eagle probably reflects their differing diets. The golden eagle's preferred diet of terrestrial herbivores such as rabbits and rodents (*7,8,9*) places it at the end of a shorter food chain than that of the bald eagle, which frequently takes fish (*10*).

Reichel et al. (6) also presented residue values for 21 golden eagle samples collected in 1964 and 1965, mostly in South Dakota. These birds also contained residues lower than those in bald eagles, with median values of 0.49 ppm DDE and 0.09 ppm dieldrin in the carcass, and 0.10 ppm DDE and less than 0.05 ppm dieldrin in the brain. The 40 golden eagle samples from South Dakota for the years 1964-70 presented in Table 3 contained similar residue levels: median values of 0.2 ppm DDE and less than 0.1 ppm dieldrin in BHKLM; less than 0.1 ppm DDE and 0.1 dieldrin in the brain.

TABLE 3.—*Organochlorine residues in golden eagles, listed by compound and State– [1]964-70*

Residues in ppm, wet-weight basis

State	BHKLM			Brain			Fat		
	Median	Range	N [1]	Median	Range	N [1]	Median	Range	N [1]
DDE									
Alaska	9.3	—	1	1.0	—	1	—	—	—
Ariz.	—	—	—	—	—	—	4.1	—	1
Ark.	—	—	—	—	—	—	0.3	—	1
Calif.	0.5	<0.1-5.2	6	0.2	<0.1-2.4	6	0.4	<0.1-16	5
Colo.	0.3	<0.1-6.5	12	<0.1	—	4	0.8	<0.1-15	10
Idaho	4.0	0.1-8.0	2	<0.1	<0.1-0.3	3	1.8	0.7-3.0	2
Ill.	0.1	—	1	—	—	—	1.6	—	1
Ind.	1.6	—	1	—	—	—	13	—	1
Iowa	<0.1	<0.1-0.1	2	—	—	—	—	—	—
Md.	3.2	0.7-5.7	2	2.3	—	1	84	—	1
Minn.	0.6	<0.1-7.9	7	0.4	<0.1-0.7	4	7.1	1.8-40	5
Mo.	0.4	0.3-3.1	3	0.8	0.2-1.4	2	<0.1	—	1
Nebr.	0.2	<0.1-2.6	26	<0.1	<0.1-1.1	11	2.5	<0.1-26.0	28
Nev.	0.3	—	1	0.1	—	1	—	—	—
N. Mex.	0.1	—	1	—	—	—	1.6	—	1
N. Dak.	0.3	<0.1-24	8	<0.1	<0.1-0.2	4	3.4	1.3-50	8
Okla.	0.5	—	1	—	—	—	10	—	1
Oreg.	—	—	—	1.2	—	1	28	1.3-55	2
Pa.	1.0	0.4-6.8	4	0.2	<0.1-0.5	4	29	6.6-38	4
S. Dak.	0.2	<0.1-25	41	<0.1	<0.1-4.6	18	4.4	1.0-28	23
Utah	0.4	<0.1-4.0	6	0.5	<0.1-1.9	3	3.6	0.3-55	4
Wis.	6.2	1.4-11	2	5.0	0.1-9.9	2	12	—	1
All States	0.3	<0.1-25	127	0.1	<0.1-9.9	65	3.0	<0.1-84	100
DIELDRIN									
Calif.	—	—	—	—	—	—	<0.1	—	1
Colo.	<0.1	<0.1-4.4	8	<0.1	—	3	0.8	<0.1-12	10
Idaho	<0.1	—	1	—	—	—	—	—	—
Ill.	0.1	—	1	—	—	—	1.3	—	1
Ind.	0.2	—	1	—	—	—	—	—	—
Iowa	<0.1	—	1	—	—	—	—	—	—
Minn.	0.1	<0.1-0.2	3	<0.1	—	2	0.7	0.4-1.9	4
Mo.	—	—	—	<0.1	—	1	<0.1	—	1
Nebr.	<0.1	<0.1-0.4	15	<0.1	<0.1-0.1	3	0.4	<0.1-2.2	22
Nev.	0.1	—	1	<0.1	—	1	—	—	—
N. Mex.	<0.1	—	1	—	—	—	<0.1	—	1
N. Dak.	<0.1	<0.1-1.5	4	<0.1	—	1	1.4	0.4-3.1	6
Okla.	<0.1	—	1	—	—	—	0.7	—	1
Pa.	0.2	—	1	—	—	—	2.4	—	1
S. Dak.	<0.1	<0.1-3.0	27	<0.1	<0.1-0.4	8	0.8	0.2-6.6	20
Utah	0.1	—	3	—	—	—	<0.1	—	1
All States	<0.1	<0.1-4.4	68	<0.1	<0.1-0.4	19	0.7	<0.1-12	69
HEPTACHLOR EPOXIDE									
Ark.	—	—	—	—	—	—	<0.1	—	1
Calif.	—	—	—	—	—	—	<0.1	—	1
Colo.	0.1	<0.1-0.2	3	<0.1	—	2	0.4	<0.1-1.2	8
Md.	—	—	—	—	—	—	1.4	—	1
Minn.	<0.1	—	1	—	—	—	—	—	—
Mo.	0.2	—	1	0.1	—	1	<0.1	—	1
Nebr.	<0.1	<0.1-0.1	11	0.1	—	1	0.7	<0.1-1.2	9
N. Dak.	0.4	—	1	—	—	—	1.1	0.5-2.4	3
S. Dak:	0.2	<0.1-12.2	6	0.3	<0.1-2.3	3	0.5	0.2-1.3	7
Utah	—	—	—	—	—	—	<0.1	—	1
All States	<0.1	<0.1-12.2	23	0.1	<0.1-2.3	7	0.4	<0.1-2.4	32

TABLE 3.—*Organochlorine residues in golden eagles, listed by compound and State—1964-70*—Continued

Residues in ppm, wet-weight basis

State	BHKLM			Brain			Fat		
	Median	Range	N [1]	Median	Range	N [1]	Median	Range	N [1]
PCB									
Calif.	2.1	<1.0-4.2	2	<1.0	—	1	9.5	—	1
Idaho	6.0	—	1	—	—	—	—	—	—
Ill.	—	—	—	—	—	—	6.4	—	1
Md.	—	—	—	<1.0	—	1	3.0	—	1
Minn.	10	<1.0-19	2	<1.0	<1.0-1.0	2	<1.0	—	1
Mo.	10	—	1	5.0	—	1	—	—	—
Nebr.	<1.0	—	3	<1.0	—	3	<1.0	—	2
N. Dak.	1.0	—	1	—	—	—	6.0	2.0-10	2
Pa.	<1.0	—	3	<1.0	—	3	<1.0	—	3
S. Dak.	<1.0	<1.0-10	4	<1.0	<1.0-0.2	3	7.0	<1.0-14	2
Utah	5.0	<1.0-10	2	—	—	—	—	—	—
Wis..	7.0	—	1	6.0	—	1	—	—	—
All States	<1.0	<1.0-19	20	<1.0	<1.0-6.0	15	<1.0	<1.0-14	13
DDD									
Ark.	—	—	—	—	—	—	0.8	—	1
Calif.	—	—	—	—	—	—	1.0	—	1
Colo.	0.2	<0.1-0.3	2	—	—	—	<0.1	—	4
Nebr.	<0.1	—	2	—	—	—	<0.1	—	1
N. Mex.	<0.1	—	1	—	—	—	1.6	—	1
Okla.	<0.1	—	1	—	—	—	0.5	—	1
Pa.	0.1	—	1	—	—	—	2.0	—	1
S. Dak.	0.2	<0.1-8.0	3	<0.1	—	1	0.9	<0.1-1.2	3
All States	<0.1	<0.1-8.0	10	<0.1	—	1	0.5	<0.1-2.0	13
DDT									
Ark.	—	—	—	—	—	—	<0.1	—	1
Colo.	0.2	—	2	—	—	—	<0.1	—	6
Idaho	—	—	—	<0.1	—	1	—	—	—
Minn.	—	—	—	<0.1	—	1	—	—	—
S. Dak.	<0.1	—	1	0.2	—	1	—	—	—
Utah	—	—	—	—	—	—	<0.1	—	1
All States	0.2	<0.1-0.2	3	<0.1	<0.1-0.2	3	<0.1	—	8
ENDRIN									
Colo.	<0.1	—	1	—	—	—	—	—	—
Md.	—	—	—	—	—	—	<0.1	—	1
Nebr.	<0.1	—	1	—	—	—	<0.1	—	3
N. Dak.	—	—	—	—	—	—	0.3	—	1
All States	<0.1	—	2	—	—	—	<0.1	<0.1-0.3	5
DDMU									
Colo.	0.6	0.5-0.6	2	—	—	—	—	—	—
ALDRIN									
Calif.	—	—	—	<0.1	—	1	—	—	—
Colo.	—	—	—	—	—	—	<0.1	<0.1-0.1	3
S. Dak.	<0.1	—	1	—	—	—	—	—	—
All States	<0.1	—	1	<0.1	—	1	<0.1	<0.1-0.1	3

[1] Number of specimens containing residue. Median is based on this number.

Dieldrin occurred in 69 (68%) of the 102 fat samples analyzed (Table 2). Only 68 BHKLM samples (51%) contained dieldrin, and 44 of these contained less than 0.1 ppm; the highest level of dieldrin was 4.4 ppm in a fat sample of a bird from Colorado (Table 3). Only 19 brain samples (26%) contained dieldrin, and 16 of these contained less than 0.1 ppm. The highest level found in the brain was 0.4 ppm in a bird from South Dakota. This is considerably lower than the minimum of 4 ppm in the brain judged necessary for acute toxic effects by Stickel et al. (*11*). In contrast, Mulhern et al. (5) found that 8 of 69 bald eagles contained sufficiently high dieldrin levels in the brain to suggest dieldrin poisoning. The levels shown here in golden eagles do not preclude sublethal effects, however, as indicated by the studies of Lockie et al. (*12*), Hickey and Anderson (*13*), and Ratcliffe (*14*).

PCB's were first quantified in golden eagles in this laboratory in 1967 (Table 4). Their presence in 20 of 113 BHKLM samples from 1967-70 indicates their ubiquitous nature.

Heptachlor epoxide, DDD, DDMU, DDT, endrin, and aldrin were seen in a few samples, usually in small quantities. The highest median levels were 0.5 ppm for DDD in fat samples and 0.6 ppm for DDMU in BHKLM sam-

TABLE 4.—*Organochlorine residues in golden eagles by year—1964-70*

Residues in ppm, wet-weight basis

Year	BHKLM			Brain			Fat		
	Median	Range	N [1]	Median	Range	N [1]	Median	Range	N [1]
DDE									
1964	1.0	0.6-1.0	3	—	—	—	0.4	<0.1-6.5	8
1965	0.8	<0.1-25	6	<0.1	—	1	6.3	0.8-26	5
1966	0.4	<0.1-6.5	11	0.1	—	2	4.2	0.3-40	7
1967	0.2	<0.1-24	39	<0.1	<0.1-0.6	6	3.0	<0.1-84	30
1968	0.2	<0.1-9.3	39	0.1	<0.1-4.6	31	2.8	<0.1-55	30
1969	0.3	<0.1-8.0	25	<0.1	<0.1-1.4	22	3.7	0.3-35	18
1970	6.4	0.8-11	4	0.7	0.2-9.9	3	33	11.0-55	2
1964-70	0.3	<0.1-25	127	0.1	<0.1-9.9	65	3.0	<0.1-84	100
DIELDRIN									
1964	0.1	—	1	—	—	—	1.2	<0.1-12	8
1965	<0.1	<0.1-3.0	4	—	—	—	0.5	<0.1-2.2	5
1966	<0.1	<0.1-4.4	10	<0.1	—	2	1.6	<0.1-2.4	5
1967	<0.1	<0.1-2.3	24	<0.1	—	1	0.5	<0.1-3.1	24
1968	0.1	<0.1-0.6	17	<0.1	<0.1-0.4	9	1.2	<0.1-6.6	17
1969	<0.1	<0.1-0.4	12	<0.1	—	7	0.7	0.2-4.6	10
1970	—	—	—	—	—	—	—	—	—
1964-70	<0.1	<0.1-4.4	68	<0.1	<0.1-0.4	19	0.7	<0.1-12	69
HEPTACHLOR EPOXIDE									
1964	—	—	—	—	—	—	0.4	<0.1-1.2	8
1965	<0.1	—	1	—	—	—	0.8	0.5-1.1	2
1966	<0.1	<0.1-0.2	4	—	—	—	1.2	<0.1-2.4	2
1967	<0.1	<0.1-12.2	10	<0.1	—	1	0.7	<0.1-1.4	13
1968	0.1	<0.1-0.6	4	<0.1	<0.1-2.3	4	0.4	<0.1-1.2	4
1969	<0.1	<0.1-0.2	4	0.1	—	2	0.2	<0.1-0.4	3
1970	—	—	—	—	—	—	—	—	—
1964-70	<0.1	<0.1-12.2	23	0.1	<0.1-2.3	7	0.4	<0.1-2.4	32
PCB									
1964	—	—	—	—	—	—	—	—	—
1965	—	—	—	—	—	—	—	—	—
1966	—	—	—	—	—	—	—	—	—
1967	<1.0	<1.0-1.0	2	<1.0	—	1	5.0	<1.0-10.0	2
1968	<1.0	<1.0-10	7	<1.0	—	6	<1.0	<1.0-6.4	7
1969	<1.0	<1.0-10	7	<1.0	<1.0-5.0	6	<1.0	<1.0-9.5	3
1970	8.0	1.0-19	4	4.0	1.0-6.0	2	14	—	1
1964-70	<1.0	<1.0-19	20	<1.0	<1.0-6.0	15	<1.0	<1.0-14	13
DDD									
1964	—	—	—	—	—	—	<0.1	—	4
1965	8.0	—	1	—	—	—	—	—	—
1966	<0.1	<0.1-0.3	3	—	—	—	1.2	0.8-1.6	2
1967	<0.1	—	3	—	—	—	0.2	<0.1-1.0	4
1968	0.1	—	1	—	—	—	2.0	—	1
1969	0.1	<0.1-0.2	2	<0.1	—	1	1.0	0.9-1.2	2
1970	—	—	—	—	—	—	—	—	—
1964-70	<0.1	<0.1-8.0	10	<0.1	—	1	0.5	<0.1-2.0	1
DDT									
1964	—	—	—	—	—	—	<0.1	—	6
1965	—	—	—	—	—	—	—	—	—
1966	0.2	—	2	—	—	—	<0.1	—	1
1967	<0.1	—	1	<0.1	—	1	<0.1	—	1
1968	—	—	—	—	—	—	—	—	—
1969	—	—	—	0.1	<0.1-0.2	2	—	—	—
1970	—	—	—	—	—	—	—	—	—
1964-70	0.2	<0.1-0.2	3	<0.1	<0.1-0.2	3	<0.1	—	8

TABLE 4.—*Organochlorine residues in golden eagles by year—1964-70*—Continued

Residues in ppm, wet-weight basis

Year	BHKLM			Brain			Fat		
	Median	Range	N[1]	Median	Range	N[1]	Median	Range	N[1]
ENDRIN									
1964	—	—	—	—	—	—	—	—	—
1965	<0.1	—	1	—	—	—	<0.1	—	1
1966	<0.1	—	1	—	—	—	—	—	—
1967	—	—	—	—	—	—	<0.1	<0.1-0.3	4
1968	—	—	—	—	—	—	—	—	—
1969	—	—	—	—	—	—	—	—	—
1970	—	—	—	—	—	—	—	—	—
1964-70	<0.1	—	2	—	—	—	<0.1	<0.1-0.3	5
DDMU									
1964	0.6	0.5-0.6	2	—	—	—	—	—	—
1965	—	—	—	—	—	—	—	—	—
1966	—	—	—	—	—	—	—	—	—
1967	—	—	—	—	—	—	—	—	—
1968	—	—	—	—	—	—	—	—	—
1969	—	—	—	—	—	—	—	—	—
1970	—	—	—	—	—	—	—	—	—
1964-70	0.6	0.5-0.6	2	—	—	—	—	—	—
ALDRIN									
1964	—	—	—	—	—	—	<0.1	<0.1-0.1	3
1965	—	—	—	<0.1	—	1	—	—	—
1966	—	—	—	—	—	—	—	—	—
1967	<0.1	—	1	—	—	—	—	—	—
1968	—	—	—	—	—	—	—	—	—
1969	—	—	—	—	—	—	—	—	—
1970	—	—	—	—	—	—	—	—	—
1964-70	<0.1	—	1	<0.1	—	—	<0.1	<0.1-0.1	3

[1] Number of specimens containing residue. Median is based on this number.

ples (for median levels in birds from all States, see Table 3). Each of these insecticides had a median of less than 0.1 ppm in the brain.

The data in Table 4 suggest that exposure of golden eagles to organochlorine insecticides has not changed significantly from 1964 through 1970. The years with the largest sample sizes (1966-69) indicate no important changes in exposure. The median concentrations of DDE, which is represented by the largest number of samples, ranged only from 0.2 to 0.4 ppm in BHKLM samples, and from 2.8 to 4.2 ppm in the fat samples during these years. This is similar to the findings reported by Mulhern et al. (5) for bald eagles.

Cases in which necropsy or residue analysis indicated the probable cause of death are listed in Table 5. Of the 63 eagles included, 33 died from shooting or contact with power lines (collision or electrocution). Six of the eagles killed by 1080 (sodium monofluoroacetate) were collected in 1966, three in 1968, and two in 1969. Most of the specimens examined died from undetermined causes, which undoubtedly included a high proportion of natural deaths.

TABLE 5.—*Probable cause of death of 169 golden eagles as determined by necropsy or residue analyses*

Probable cause of death	Number
Shooting	18
Impact on power line or electrocution	15
1080 (sodium monofluoroacetate)	11
Trapping	10
Collision with vehicle	5
Strychnine	2
Cyanide	2
Unknown	106

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Toxaphene Content of Estuarine Fauna and Flora Before, During, and After Dredging Toxaphene-Contaminated Sediments[1]

Robert J. Reimold and Charles J. Durant

ABSTRACT

This paper evaluates toxaphene concentrations in selected estuarine fauna, flora, sediment, and dredge spoil before, during, and after the dredging of Terry Creek, Brunswick, Ga., in autumn 1972. This is the second effort to widen the channel of the creek, which receives the effluent from a nearby toxaphene-manufacturing plant; a 1971 dredging effort was aborted by the State of Georgia. The current study employed safeguards inspired by the 1971 State action: enclosure of dredge spoil in diked areas of unproductive marshland to prevent runoff, and weekly monitoring of Terry Creek biota and sediment to detect the possible role of toxaphene in any resulting disturbance to the balance of nature. Monitoring of dredge spoil, fauna, and flora showed toxaphene concentrations to be higher during dredging than before or after. Eastern oysters (Crassostrea virginica), *reported to be among the best biological monitors, did not demonstrate large changes in toxaphene content resulting from the dredging. The high toxaphene concentration in oysters ranged between 2.0 and 5.0 ppm. The best indicators evaluated were salt marsh cordgrass* (Spartina alterniflora) *and mummichog* (Endulus heteroclitus). *The highest content found in* S. alterniflora *was 7.5 ppm; the highest concentration in* F. heteroclitus *was over 200 ppm.*

Introduction

A unique occasion to study the dispersal of the chlorinated hydrocarbon, toxaphene, and the relation of its concentration to waterway maintenance dredging arose near Brunswick, Ga. Terry Creek, which receives the effluent from a toxaphene-manufacturing plant, was the site of a recent dredging operation. An earlier operation (*1*) had been aborted after 100 yards of dredging, when the State of Georgia objected that toxic products in the undiked spoil sediment might drain through the marshland on which the spoil was being deposited. Such drainage would contaminate both the potentially productive marshland and the estuarine ecosystem into which it drained. The current study initiated safeguards against such a contingency: enclosure of dredge spoil in diked areas of marshland which had been identified by remote sensing techniques (*2,3*) as less productive than the marshland initally used as deposit sites; and weekly monitoring of Terry Creek biota and sediment to detect the possible role of toxaphene in any resulting disturbance to the balance of nature.

The monitoring program which the U.S. Army Corps of Engineers, Savannah District, outlined in response to the State of Georgia was designed to document at weekly intervals the possible role of toxaphene in any ecologic disturbance resulting from the dredging of Terry Creek. Toxaphene concentrations were measured in selected estuarine fauna, flora, sediment, and dredge spoil during dredging. Findings were compared to the authors' earlier baseline studies of pesticide concentrations in the estuarine ecosystem, which revealed toxaphene in many portions of the food web and highly concentrated in the sediments of the creek (*4*).

Methods

Terry Creek was dredged by hydraulic pumping and 5×10^6 cubic yards of spoil was placed in two diked

[1] Contribution No. 30, University of Georgia Marine Institute, Sapelo Island, Ga. 31327.

areas on a nearby salt marsh (areas C and D, Fig. 1). Collection sites for environmental samples are indicated in the same figure: A) Back River Bridge; B) mouth of Terry Creek; C) west dredge spoil area near the toxaphene plant outfall; D) east dredge spoil area near Back River; and E) main channel of Terry Creek. Field collections were obtained at weekly intervals beginning one week before dredging operations started on September 7, 1972, and terminating 8 weeks later, 1 week after the completion of all dredging on October 26, 1972. Additional baseline data on the toxaphene content of Terry Creek organisms were collected during 1970 and 1971 and followup collections continued at monthly intervals for several months. Environmental samples included finfish collected by otter trawl and cast net in the main channel of Terry Creek; salt marsh cordgrass (*Spartina alterniflora*) collected near the dredge spoil dike areas; sediment collected from the mouth of Terry Creek and from within each of the diked spoil areas; and oysters (*Crassostrea virginica*) collected from Back River Bridge.

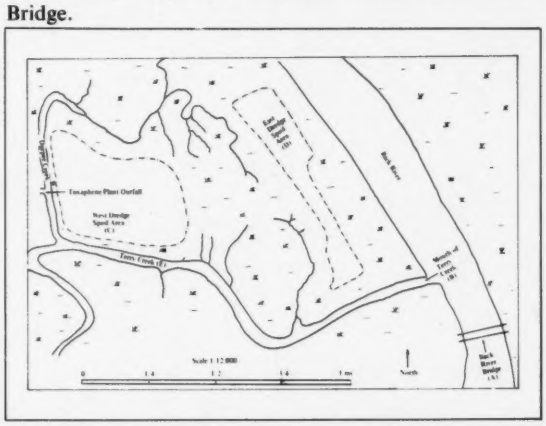

FIGURE 1.—*Geographic location of collection sites near Terry Creek, Brunswick, Ga., 1972*

Samples were processed and analyzed for toxaphene according to authors' methods in the earlier baseline studies (*4*) and the technique of Wilson (*5*). Specific details are summarized in Table 1. Chromatograms of environmental samples were so similar to chromatograms of technical grade toxaphene standard that no polychlorinated biphenyls were indicated. All concentrations are expressed in parts per million (ppm) wet weight except *S. alterniflora* which is based on dry weight. Prior to analysis, *S. alterniflora* plant parts were given four rinses in tap water to remove surface salt deposits. Each leaf and stem was then blotted dry with tissue to remove residual rinse water. The relative recovery from oysters and sediment was above 85 percent and 90 percent, respectively. Data were not corrected for this error and concentrations below 0.25 ppm were considered insignificant with the exception of those included in water samples. Concentrations less than 0.0010 ppm were considered below the limits of detection.

TABLE 1.—*Summary of column packing and operating parameters used for toxaphene analysis*

Column:	5 ft by ⅛ in., glass, packed with 3% DC-200 on Gas Chrom Q, 80/100 mesh
Temperature:	Detector 210°C Injector 210°C Oven 190°C
Carrier:	Prepurified nitrogen at a flow rate of 40 ml/min

Results

Results of toxaphene analyses in fauna, flora, and sediment reveal that toxaphene was generally higher in dredge spoil sediment than in any other samples processed (Table 2). Comparisons of concentrations in mummichog (*Fundulus heteroclitus*), white shrimp (*Penaeus setiferus*), marsh grass, and sediment show that, except for dredge spoil within the diked areas, concentration rarely exceeded 10.0 ppm (Fig. 2-10).

Butler (*6*) demonstrated that shellfish can produce biological magnification of pesticide levels up to 70,000 times those of surrounding water. Yet the levels in oysters collected at Back River Bridge (Fig. 1) adjacent to the mouth of Terry Creek never exceeded 2.0 ppm during the dredging operation.

Toxaphene concentrations in dredge spoil from the western enclosure close to the toxaphene plant outfall (Fig. 1) neared 1,000 ppm (Fig. 5). The highly contaminated sediment in both the western and eastern enclosures was held within two dikes (Fig. 1) and did not appear to influence the surrounding biota.

Background toxaphene concentrations in 1970-71 collections of fauna, flora, and sediment exhibited higher values than 1971-72 concentrations. The latter values remained about the same before and after dredging, reflecting in part the pollution abatement practices initiated at the toxaphene production plant which greatly decreased the quantities of toxaphene in the plant effluent.

Discussion

Toxaphene contamination appears to be best indicated by the marsh grass (*S. alterniflora*). Various species of fish are also good indicators of the increased toxaphene in the suspended material and water. Although Butler (*6*) has suggested that shellfish are among the best biological monitors for pesticide residues, the results of this study suggest that marsh grass and finfish accumulate toxaphene to a greater degree than oysters (*Crassostrea virginica*) when all are collected from the same geo-

TABLE 2.—*Toxaphene concentrations, ppm, found during fall 1972 monitoring of Terry Creek dredging operation*[1]

	Replicate No.	Cruise No. 1 (Sept. 2)		Cruise No. 2 (Sept. 14)	Cruise No. 3 (Sept. 21)	Cruise No. 4 (Sept. 28)	Cruise No. 5 (Oct. 5)	Cruise No. 6 (Oct. 12)	Cruise No. 7 (Oct. 19)		Cruise No. 8 (Oct. 26)
Mummichog (*Fundulus heteroclitus*)	1	10.45	DREDGING BEGINS: Sept. 7, 1972	NSC	NSC	10.52	NSC	131.14	12.07	DREDGING ENDS: Oct. 20, 1972	NSC
	2	8.97		NSC	NSC	3.41	NSC	217.14	5.18		NSC
Salt marsh cordgrass (*Spartina alterniflora*)	1	0.82		1.13	0.73	2.04	0.81	3.93	7.33		1.69
	2	0.76		1.56	0.56	2.54	0.91	3.68	6.26		2.92
Eastern oyster (*Crassostrea virginica*)	1	1.20		1.42	1.55	1.21	1.26	1.19	1.70		0.94
	2	1.37		1.33	1.71	1.15	1.23	1.24	1.79		0.95
Sediment (near entrance to Terry Creek)	1	5.47		5.56	0.87	2.11	5.55	3.97	2.11		2.64
	2	4.42		4.20	0.94	2.68	7.24	4.06	2.23		3.74
Dredge spoil from east enclosure (Back R.)	1	NSC		0.81	60.08	5.70	NSC	NSC	NSC		NSC
	2	NSC		0.79	150.79	5.12	NSC	NSC	NSC		NSC
Dredge spoil from west enclosure (Dupree C.)	1	NSC		NSC	NSC	NSC	93.39	756.40	51.64		241.64
	2	NSC		NSC	NSC	NSC	119.52	812.64	142.42		331.55
Water sample	1	ND		NSC	0.0013	0.0013	0.0016	0.0014	NSC		NSC
	2	ND		NSC	NAS	NAS	NAS	NAS	NSC		NSC
Anchovy (*Anchoa mitchelli*)	1	NSC		8.61	NSC	20.46	8.96	9.89	12.85		10.51
	2	NSC		NAS	NSC	16.60	NAS	10.13	11.96		NAS
Shrimp (*Penaeus setiferus*) head and thorax	1	NSC		1.21	4.80	3.22	3.03	2.88	4.19		10.69
	2	NSC		1.51	4.65	2.24	2.85	NAS	4.30		2.41
Shrimp (*Penaeus setiferus*) abdomen (edible tail)	1	NSC		0.58	0.86	1.22	0.92	1.49	0.74		2.51
	2	NSC		0.58	0.90	0.88	0.73	NAS	0.64		0.83
Star drum (*Stellifer lanceolatus*)	1	NSC		2.43	2.09	NSC	2.72	NSC	NSC		1.42
	2	NSC		2.61	3.24	NSC	2.48	NSC	NSC		1.09

[1] NSC—no sample collected; NAS—not adequate sample; ND—not detectable.

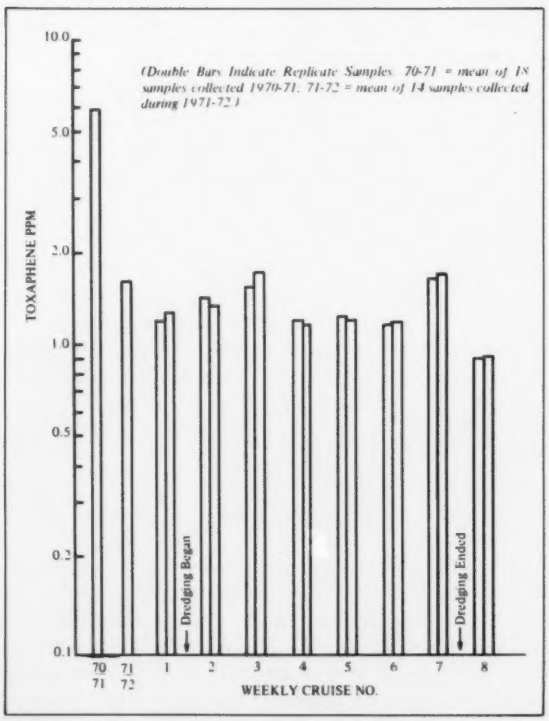

FIGURE 2.—*Toxaphene concentration, ppm, in oysters* (Crassostrea virginica) *from Back River Bridge, Terry Creek, Brunswick, Ga., 1972*

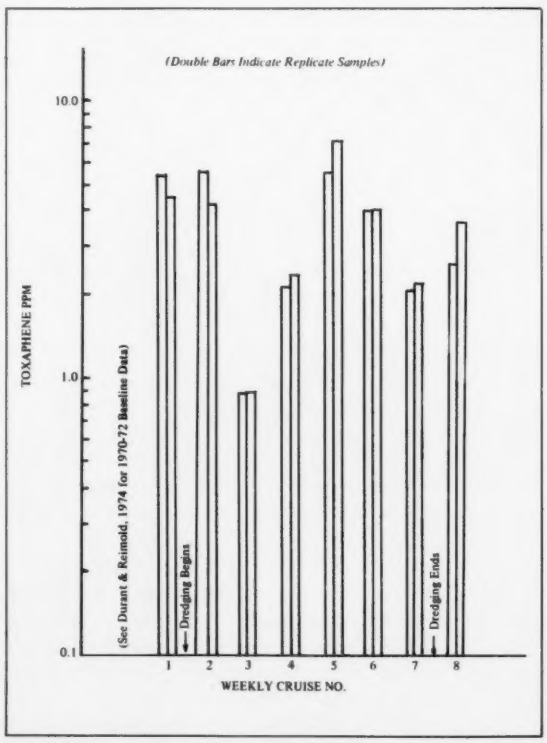

FIGURE 3.—*Toxaphene concentration, ppm, in surface sediment from mouth of Terry Creek, Brunswick, Ga., 1972*

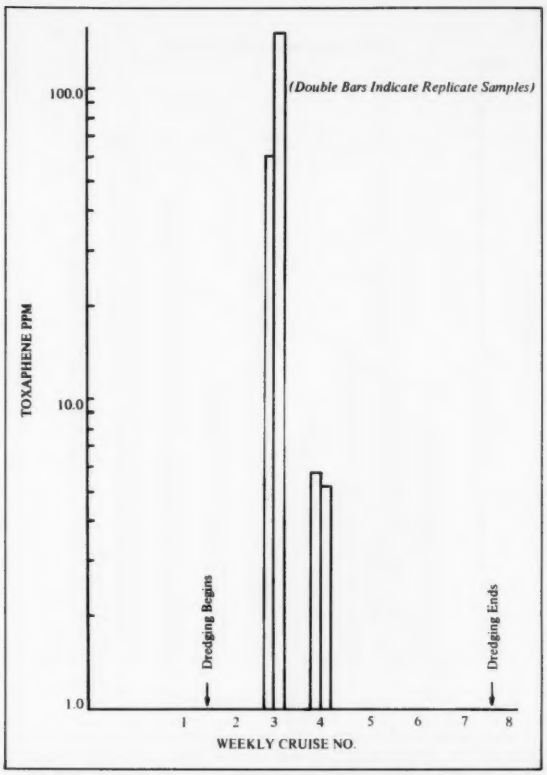

FIGURE 4.—*Toxaphene concentration, ppm, in dredge spoil from east enclosure near Terry Creek, Brunswick, Ga., 1972*

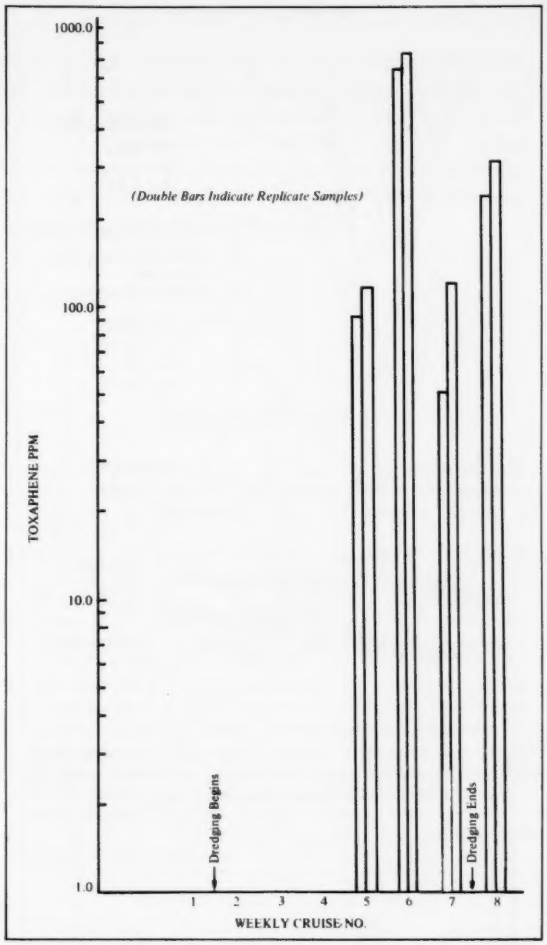

FIGURE 5.—*Toxaphene concentration, ppm, in dredge spoil from west enclosure near Terry Creek, Brunswick, Ga., 1972*

graphic location. The authors submit that different organisms may be able to preferentially concentrate different residues. Although relatively high concentrations of toxaphene were found in anchovy (*Anchoa mitchelli*), it was frequently absent from the trawl catch.

The high toxaphene content of *S. alterniflora* is of interest since it represents a translocation of toxaphene from the sediments into the plant tissue. *S. alterniflora* is a halophyte and probably translocates toxaphene along a pathway similar to the movement of salt. This is unique because it is one of the only known instances of toxaphene translocation in high concentrations among primary producers.

Altogether, over 125 samples were analyzed for toxaphene during the 8-week study. In addition, qualitative visual examination of the Terry Creek area was made at weekly intervals to assess potential fish kill. Fewer than 20 dead fish were recovered during the 8 collection trips of 4 to 5 hours each. Those recovered and positively identified included tongue-fish (*Symphurus sp.*) harvest fish (*Peprilus sp.*), silver perch (*Bairdiella sp.*), sea catfish (*Arius sp.*), and one menhaden (*Brevoortia sp.*). Local shrimp boats that dock in Terry Creek frequently offloaded and sorted catches during the study; this may well have been the source of dead fish, especially since the authors' sampling indicated that the species were not indigenous to Terry Creek. At no time were fish observed to be dying (alive but floating on the surface on their sides), nor did they show other signs of distress during the nearly 100 person-hours of field sampling in Terry Creek.

It is the conclusion of this study that dredging did not dramatically alter the biological balance of the estuary by toxaphene contamination when toxaphene residues were isolated in diked enclosures. As reported by the authors in the baseline studies (*4*), the combination of ultraviolet radiation and biological degradation should render the impounded sediments nontoxic in a few years.

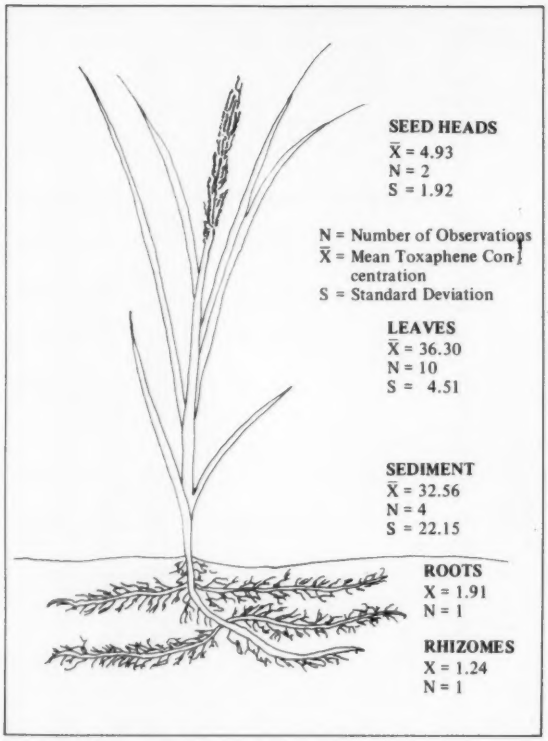

FIGURE 6.—*Toxaphene concentration, ppm, in marsh grass* (Spartina alterniflora) and surrounding sediments collected *from Terry Creek Marsh, Brunswick, Ga., 1970-72*

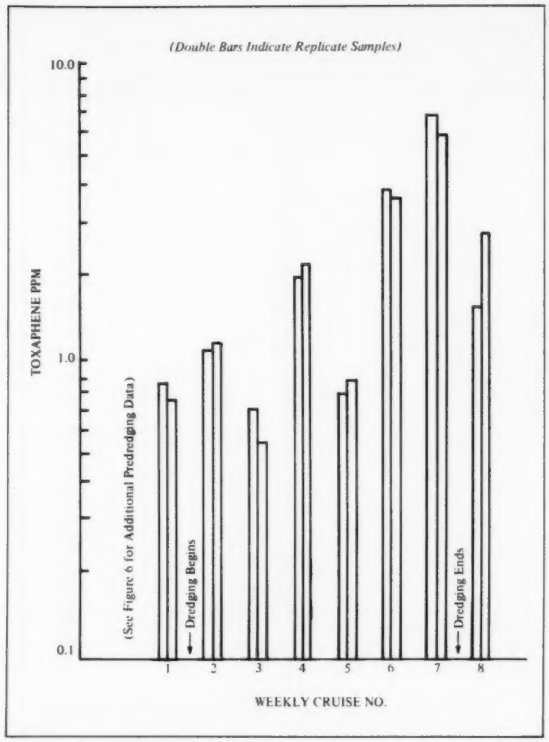

FIGURE 7.—*Toxaphene concentration, ppm, in marsh grass* (Spartina alterniflora) *leaves and stems from bank of Back River near Terry Creek, Brunswick, Ga., 1972*

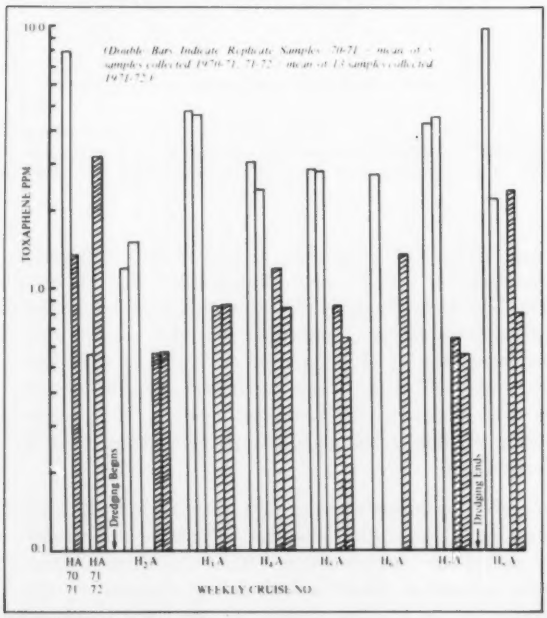

FIGURE 8.—*Toxaphene concentration, ppm, in white shrimp* (Penaeus setiferus) *from Terry Creek, Brunswick, Ga., 1972*

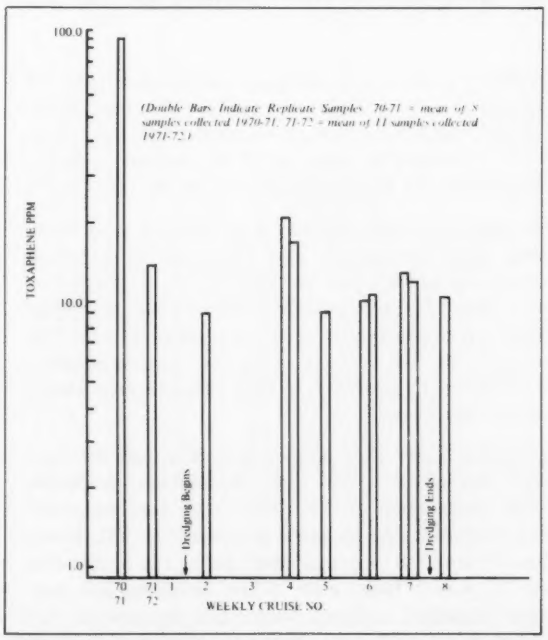

FIGURE 9.—*Toxaphene concentration, ppm, in anchovy* (Anchoa mitchelli) *from Terry Creek, Brunswick, Ga., 1972*

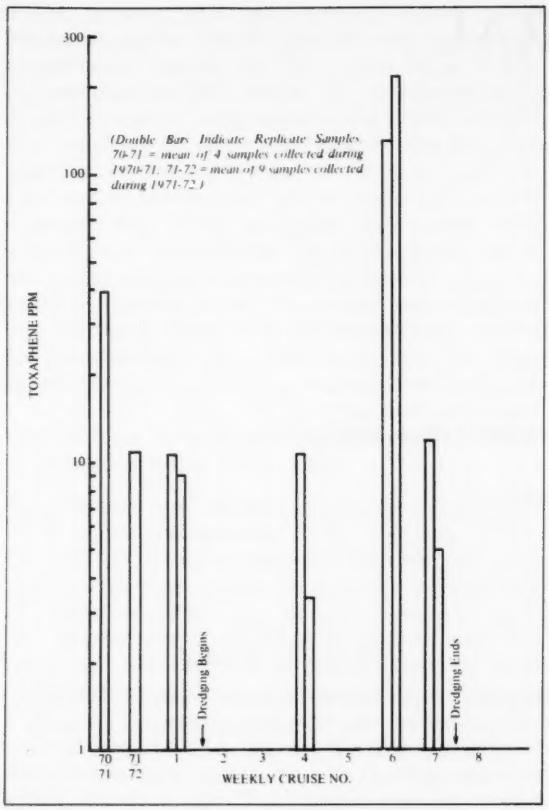

FIGURE 10.—*Toxaphene concentration, ppm, in mummichog* (Fundulus heteroclitus) *from Terry Creek, Brunswick, Ga., 1972*

Acknowledgments

This research was supported by a grant from Hercules, Inc., to the University of Georgia Marine Institute and a contract between the Savannah District U.S. Army Corps of Engineers and the University of Georgia Marine Institute. The authors also wish to acknowledge the assistance of J. E. Durant and P. C. Adams for analysis and collection of the data.

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GENERAL

Resmethrin Residues in Foliage after Aerial Application

Theresa L. Andrews [1]

ABSTRACT

The residue present after aerial application of 0.05 and 0.15 lb per acre of the synthetic pyrethroid, resmethrin, was analyzed on three kinds of foliage and in water samples. The compound was identified by thin-layer chromatography (TLC) detecting from 0.06 to 0.25 ppm. The residue on aspen and Douglas fir showed that the initial deposit was light, and no detectable amount was found after seven days. The highest residue, 1 ppm, was found on willow taken the day of insecticide application. No detectable quantities were found in the water samples.

Introduction

As part of the U.S. Department of Agriculture Forest Service program to evaluate potential insecticides, a preliminary safety evaluation was conducted on the synthetic pyrethroid, resmethrin. The study was done on the Pike National Forest near Colorado Springs, Colorado, in cooperation with the U.S. Department of Interior Bureau of Sport Fisheries and Wildlife. This continuing program is designed to ascertain the hazards of insecticides to nontarget organisms such as birds, aquatic and terrestrial invertebrates, and fish.

This paper describes the method of detection, amount of deposit, and persistence of the insecticide resmethrin after spray application by helicopter at two dosage levels. This chemical was found to possess a high degree of activity on several forest insects (*1*).

[1] Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Berkeley, Calif. 94701.

Methods and Materials

Resmethrin is the proposed common name for 5-benzyl-3-furyl-methyl (± -cis, trans-chrysanthemate), a mixture of synthetic pyrethroid first described by Elliot, et al. (*2*). This mixture of isomers has been called NRDS 104, SBP-1382, and NIA 17370. It is available commercially from S. B. Pennick and Co., New York, as SBP-1382.

Natural pyrethrins are easily analyzed by electron-capture gas chromatography, but resmethrin cannot be detected in this manner. The hydrogen flame detector could be used to detect 3 μg of resmethrin. However, it was impossible to detect this quantity from plants because the extractives interfered with the hydrogen flame detection system.

Early investigation of the photostability of resmethrin was carried out by using Rhodamine B as a chromogenic detector on thin-layer chromatography (TLC). The greatest sensitivity was achieved with Isatin in sulfuric acid as a chromogenic agent. This chromogen was originally used to detect thiophene (*3*), and was developed for the detection of resmethrin at a 0.1-μg level by Abernathy, et al. (*4*). Isatin sprayed on 10 μl of control solutions of resmethrin in ethyl acetate (w/v) containing 0.1, 0.2, 0.5, 0.75, 1.0, and 2.5 μg produced a stable, measurable, and readily detectable red spot on TLC plates. However, plant extracts produced interfering color in the presence of hot sulfuric acid. By moving the interfering materials to the front of the TLC with n-hexane, resmethrin could be identified on silica gel G (250 μ).

Twenty grams of local plant material (willow, poplar, Englemann spruce, and Douglas fir) were treated with resmethrin at rates of 1.0, 5.0, 10, and 20 μg as preliminary plant fortified controls. The stems, needles, or leaves were cut into small segments and placed in Mason jars containing 20 ml of ethyl acetate. The jars were placed in an enclosed dry ice bin for 10 minutes. The ethyl acetate was decanted, and the frozen material was macerated with mortar and pestle and washed twice with 20 ml of ethyl acetate. The ethyl acetate fraction was evaporated under vacuum to around 5 ml and transferred to centrifuge tubes. The volume was evaporated with nitrogen to about 0.5 ml and centrifuged to remove the small particles. The clear supernatant was transferred, and the precipitate was washed three times with 1 ml of ethyl acetate. The samples were evaporated under nitrogen to the desired volume of 100 μl of which 10 μl was spotted on a TLC plate.

These samples were chromatographed in duplicate with 10 μl of the corresponding control standards of 0.1, 0.5, 1.0, and 2.0 μg of resmethrin on each plate. The plates were first developed with n-hexane to remove any interfering materials. After the plates were air-dried, two different solvent systems were used to move the resmethrin: one consisting of benzene, and the other of chloroform, ether, and ethyl acetate (2:1:2). After the plates were developed, they were sprayed with 0.5% isatin in concentrated sulfuric acid (w/v). These fortified plant samples gave a red spot equal in color intensity and size to the 10 μl quantities of the control standard solutions. The Rf values for resmethrin were 0.50 ± 6% for benzene and 0.85 ± 6% for the chloroform, ether, ethyl acetate solution.

Resmethrin was applied by a helicopter on two separate plots at two dosage levels: 0.05 lb and 0.15 lb per 0.5 g of mineral oil per acre. Field samples of willow, aspen, and Douglas fir were collected from three different areas in the plots at three different times: immediately after spray application, three days later, and one week later. Samples of water were also taken from streams in these plots. All samples were immediately placed with dry ice until extracted.

Forty grams of each of the field plant samples were extracted and handled the same as the previous plant controls. All samples including controls were spotted on a TLC plate in a 10 μl volume. If quantities less than 0.06 ppm were found in 10 μl, no further concentration by evaporation of the plant extract was performed. If quantities greater than 0.25 ppm were found, then solutions were diluted so that 10 μl contained from 0.25 to 1.0 μg resmethrin.

Controls of 100 grams of laboratory tap and distilled water were fortified with 10 or 20 μg of resmethrin. Duplicate samples were placed in sealed quart jars in a dry ice bin for periods of one hour, and for one, three, and seven days. The pH of the water varied from 6.7 to 6.9. These samples were washed three times with ethyl acetate in the same manner as the plant material. Since resmethrin is insoluble in water, no difficulties were encountered with the extraction procedure using 500 μl of ethyl acetate. The ethyl acetate was evaporated to a volume of 100 μl of which 10 μl was spotted on a TLC plate.

The field samples of water were taken from the lower, middle, and upper parts of a stream in each plot. Forty grams of each water sample was extracted three times with 500 ml of ethyl acetate. The samples were analyzed in duplicate in the same manner as the water controls. The small amount of debris in the field stream samples presented no problems during extraction. The pH varied from 5.4 to 5.8.

Results and Discussion

Immediately after the resmethrin was sprayed, no detectable residues were found on the aspen at the lower dose rates, but at the 0.15-lb rate, 0.8 ppm was found (Table 1). Only the willow samples showed a deposit as high as 1 ppm. The initial deposit was light; after seven days, no detectable residues were found. The <0.05 ppm designation was used when very faint traces were visible on the TLC. The results suggest that after aerial application of resmethrin at 0.05 and 0.15 lb/acre, no substantial residue remains after seven days.

TABLE 1.—*Resmethrin residues, ppm, found days after aerial application on three types of plant foliage*

Plant	Days after application			Control fortified with 0.5 ppm resmethrin [1]
	0	3	7	
RATE: 0.05 LB/ACRE				
Aspen	0	0	0	—
	0	0	0	—
	0	0	0	0.4
Willow	1.0	0.3	<0.05	—
	1.0	0.3	0	—
	1.0	0.3	<0.05	0.5
Douglas fir	0.3	0	0	—
	0.3	0	0	—
	0.8	0	0	0.4
RATE: 0.15 LB/ACRE				
Aspen	0.8	0	0	—
	0	0	0	—
	0.4	0	0	0.5
Willow	0.8	0.5	<0.05	—
	0.5	0	0	—
	0.5	0.4	<0.05	0.5
Douglas fir	0.3	0	0	—
	0.5	0	0	—
	0.5	0	0	0.4

[1] Average of six samples analyzed with an accuracy of ±7%.

Recovery of the 10 and 20 μg of the fortified water controls with resmethrin on the TLC plates were measured and remained unchanged within a 10 percent error. The water samples from the field showed no detectable quantities of resmethrin. Although the lesser application rate of 0.05 lb/acre of resmethrin had a fatal effect on most of the aquatic insects in the 1-hour postspray period, no toxic effects were observed in fish held in cages in the stream (5). The fact that no resmethrin was recovered from the aquatic samples even though insect mortality was high obviously indicated substantial degradation, considerable dilution of the insecticide, or insufficient understanding of resmethrin within the environment.

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Detection of DCPA Residues in Environmental Samples

F. M. Miller and E. D. Gomes

ABSTRACT

DCPA was detected in river water, five species of fish, and air in the lower Rio Grande Valley of Texas. Its presence was determined by electron-capture gas-liquid chromatography and confirmed by several analytical methods. Analyses of water samples taken over a 2-year period usually indicated less than 1 ppb DCPA in water during both years. Residues in one freshwater and four saltwater fish species varied from less than 1 ppb to 8 ppm. DCPA was found in air samples for several months following use in a vegetable-growing area.

Introduction

DCPA (Dimethyl tetrachloroterephthalate) is a herbicide that is sold under several brand names for control of grasses and weeds in turf and gardens and for use in commercial agriculture. In the lower Rio Grande Valley, DCPA is used primarily as a pre-emergence herbicide on onion soils and to a lesser extent on cabbage and cotton crops.

This chlorinated hydrocarbon pesticide is of low solubility, is adsorbed by organic matter in soil, and does not leach in any of the general soil types. Its average half-life is 100 days in most soil types and it is relatively nontoxic to animal species that have been tested (*1*).

During 1969 onion soils were sampled in 10 states: 46.5 percent had traces (0.94 ppm) of DCPA. This is in contrast to a nationwide sampling of croplands during 1969 in which only 0.1 percent of the 1,729 samples tested showed traces of DCPA (*2,3*). The herbicide has also been detected in air samples from an onion-growing area in Colorado (*4*).

This report, which presents data from several studies, indicates rather widespread environmental contamination by a chlorinated pesticide. Aquatic sampling of the two major streams in this area was initiated in 1971 to detect differences in pesticide contamination over a period of time. In 1972 selected tissues from two large samples of speckled trout were analyzed to determine the level of chlorinated hydrocarbon pesticides present. Air samples were analyzed for pesticides during 1972 as a part of a national monitoring program (*5*). Several miscellaneous samples were taken after analytical confirmation of DCPA. Because contamination of streams and fish has not been reported previously, all available data are presented in this paper. All studies were conducted in the lower Rio Grande Valley of Texas, an intensely agricultural area.

Methods and Materials

SAMPLING

Two sampling sites, one on each of the two major waterways in the lower Rio Grande Valley, were monitored in 1971 for chlorinated hydrocarbon pesticides. One sample of unfiltered water, bottom sediment, and menhaden (*Brevoortia* sp.) was taken each month from the Arroyo Colorado at a brackish water location near the port which is east of Harlingen (Fig. 1). Only water and bottom sediment samples were taken at the second Rio Grande site near Los Indios. Bottom sediment and water samples were taken in solvent-rinsed containers near the bank; the menhaden were collected by cast netting near the middle of the stream.

In 1972 two large samples of spotted seatrout (*Cynoscion nebulosus*) were taken by bait casting in the Three

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FIGURE 1. *Map of Lower Rio Grande Valley showing sampling sites for DCPA*

Islands area of the lower Laguna Madre. One sample was taken during May and the other was collected in July. The length, weight, and sex of each fish were recorded. Each speckled trout was dissected and the ovaries or testes, liver, and 10 g of edible flesh were removed and frozen until analyzed. Several Rio Grande perch (*Cichlasoma cyanoguttatum*) were collected at various freshwater locations along the Arroyo Colorado (Table 1, Fig. 1), and were dissected in the same manner to obtain similar tissues when possible. Collectors caught one mullet (*Mugil* sp.) while gathering menhaden. Two speckled trout and one red drum (*Sciaenops ocellata*) were collected and analyzed for residues of DCPA during 1973.

Air samples were taken from two locations in each of two cities from February 1972 until January 1973. One MisCo high-volume sampler was placed near the center and one on the periphery of each city. Approximately 61 cubic meters of air were sampled at each location over a 24-hour period. The combined samples from each city were analyzed as one sample. Weekly samples were taken alternately from each city.

ANALYTICAL PROCEDURES

A 1-liter water sample was extracted with 150 ml of hexane in a 2-liter separatory funnel. The contents were shaken vigorously for 2 minutes with venting and allowed to settle until the layers separated. The hexane layer was removed and another 150-ml portion of hexane was added to the water sample. Then the procedure was repeated. The two 150-ml extracts were combined and a small amount of sodium sulfate was added to absorb any water remaining in the extract. The sample was transferred to a 500-ml Kuderna-Danish evaporative concentrator and concentrated to 5 ml followed by Florisil cleanup (*6*).

Each 25-g bottom sediment sample was placed in a 250-ml Erlenmeyer flask, 150 ml of acetonitrile was added, and the sample was shaken for 1 hour at 3 agitations per second on a wrist-action shaker. The entire sample was filtered through a solvent-rinsed glass wool filter into a 1-liter separatory funnel containing 500 ml of water. The soil remaining on the filter was rinsed twice with 25 ml of acetonitrile. The acetonitrile/water mixture was partitioned twice with petroleum ether. The petroleum/ether extract was concentrated and passed through Florisil for cleanup.

Fish samples were analyzed by taking approximately 25 g of menhaden, 10 g of trout flesh, 5 g of perch flesh, and the entire ovaries, testes, or liver. Each sample was weighed and placed in a stainless steel Omni-mixer cup, 50 ml of acetonitrile were added, and the sample was blended at medium speed for 5 minutes. The ovaries were ground in a mortar with sodium sulfate and petroleum ether. The ground or blended extract was filtered through a solvent-rinsed, glass wool filter into a 1-liter separatory funnel. The sample filtercake was rinsed with 25 ml of acetonitrile; to this was added 130 ml of petroleum ether and the mixture was shaken for 2 minutes. Then 600 ml of water was added, the sample was shaken again for about 1 minute, the 2 layers were allowed to separate, and the aqueous layer was discarded. Approximately 200 ml of water was added again. The sample was shaken; the layers were allowed to separate; and the water was discarded again, removing the remaining acetonitrile. The petroleum ether extract was concentrated and cleaned on a Florisil column.

Air samples were collected by drawing atmospheric air through ethylene glycol. The samples were extracted with hexane and were cleaned on a Florisil column (*7*).

When Florisil cleanup was employed, DCPA eluted in the 15 percent fraction. This fraction was concentrated to 5 ml and analyzed by gas-liquid chromatography (GLC).

Routine pesticide analyses were accomplished with Micro-Tek 220 gas chromatographs that were equipped as follows:

Detector:	electron-capture tritium foil; 20 volts.	
Columns:	1.5% OV-17/1.95% QF-1 on Chromosorb W 100/120 mesh H.P.; 4% SE-30/6% QF-1 on Chromosorb W, H.P.; 1.6% OV-210/6.4% OV-17 on Gas Chrom Q; 5% OV-210 on Gas Chrom Q.	
Temperatures:	Inlet	225°C
	Detector	205°C
	Columns	OV-17/QF-1, SE-30/QF-1, and OV-210/OV-17 at 200°C OV-210 at 175°C.
Carrier gas:	Nitrogen.	
Flow rate:	OV-17/QF-1, 80 cc/min; OV-17/OV-210, 65 cc/min; SE-30/QF-1, 75 cc/min; OV-210, 60 cc/min.	
Sensitivity:	½ FSD with 50 pg of aldrin.	
Electrometer setting:	10x16.	

TABLE 1.—*DCPA Residues in Three Species of Fish*

Date Collected	Site of Collection	Species	Tissue	DCPA Residue, ppb
November 1972	Arroyo Colorado, Harlingen	Mullet	skin	555
			flesh	159
			viscera	231
December 1972	Arroyo Colorado, Harlingen	Rio Grande Perch	liver	468
			ovaries	420
			flesh	217
		Rio Grande Perch	liver	388
			ovaries	212
			flesh	89
February 1973	Arroyo Colorado, La Feria	Rio Grande Perch	liver	215
			ovaries	107
			flesh	29
February 1973	Arroyo Colorado, Mercedes	Rio Grande Perch	liver	190
			flesh	0
March 1973	Arroyo Colorado, Mercedes	Rio Grande Perch (1 sample: 2 small fish)	flesh	90
March 1973	Three Islands, Laguna Madre	Redfish	liver	18
			testes	132
			flesh	0

Recovery studies were conducted with unfiltered arroyo water and trout flesh. Mean DCPA recovery from water samples was 97 percent, with 88 percent recovery from fish flesh. All results are presented as unadjusted values.

CONFIRMATORY TECHNIQUES

In addition to GLC analyses, three other confirmatory techniques were used. The extracts of several arroyo water samples (20 gallons) were pooled and the presence of DCPA was confirmed by infrared analysis. The KBr micropellet was scanned on a Perkin-Elmer 337 Grating Infrared Spectrophotometer.

Several fish and water samples were analyzed by a thin-layer chromatography/GLC technique (TLC-GLC) (*7*). Since there was not enough DCPA to develop on the TLC plate, the Rf value was determined. A portion of the Al_3O_2 on the TLC plate was removed at the indicated point, extracted with hexane, and analyzed on the gas chromatograph.

Two samples were analyzed by GLC/mass spectrometry (GLC-MS) (*8*). The first sample, a pooled extract of menhaden, was cleaned up by thin-layer chromatography. When the fish extract showed many interfering substances, a second and much cleaner sample was obtained by extracting 10 gallons of arroyo water.

Results and Discussion

Since 1971 routine samples of soil, water, and menhaden have been taken from the Arroyo Colorado and analyzed by GLC. Some of the unfiltered water samples collected in 1971 showed a very prominent peak which eluted at the same retention time as that of heptachlor epoxide on OV-17/QF-1 and SE-30/QF-1 columns. Analyses of samples in the past have indicated little heptachlor or heptachlor epoxide in the Rio Grande Valley. A closer look at this mystery peak revealed a difference of 0.05 relative retention time units from that of heptachlor epoxide on the OV-210/SE-30 column. At that time, the peak was tentatively identified as DCPA. The 1972 water samples showed greater concentrations of the chemical during the fall of the year. Air samples also revealed a substance with an identical retention time. During this same time period, some of the menhaden and trout being analyzed were demonstrating this peak in tissue, liver, and reproductive organs, indicating some persistence of the chemical in the aquatic and estuarine environment. Confirmatory procedures were initiated during the latter months of 1972.

CONFIRMATORY PROCEDURES

In 1972, the OV-210 GLC column proved to be of great importance in identifying DCPA. The most critical differentiation was between heptachlor epoxide and DCPA, which elute with almost the same relative retention time on columns other than the OV-210. Most of the commonly used chlorinated hydrocarbon pesticides elute in the same general sequence when using columns such as the OV-210/OV-17 (Fig. 2). With the OV-210 column, however, the relative retention times of some chlorinated hydrocarbons differ. Heptachlor epoxide increases slightly, but the retention time of DCPA is much greater than that of heptachlor epoxide (Fig. 2).

Several samples were analyzed by flame photometric detection, indicating that the substance was not a phosphorus- or sulfur-bearing compound. The TLC-GLC technique confirmed DCPA in several fish and water samples.

A relatively pure sample of DCPA was difficult to obtain, but its presence in arroyo water was confirmed by infra-

red spectrophotometry (Fig. 3). The subnanogram amounts of DCPA in arroyo water necessitated the extraction of 20 gallons of the water in order to collect enough DCPA for infrared analysis. GLC-MS analyses confirmed the presence of DCPA in water and fish samples.

WATER AND SEDIMENT

In 1971 DCPA was identified in unfiltered water samples by GLC (Table 2). Trace amounts were found only in water, and were not confirmed by other techniques. Similar low residue levels were found throughout 1972 and 1973. Although the monthly sample in September 1972 was negative, an additional sample taken on the same date at a nearby location revealed the highest recorded water value of 1.32 ppb. Another unfiltered sample taken at the Harlingen site later in September contained 0.91 ppb DCPA. Likewise, two additional samples taken during another negative month, December 1972, contained 0.35 and 0.40 ppb DCPA. It is obvious that monthly sampling does not give an accurate estimate of the amount of DCPA in the stream. Only one DCPA residue of 0.08 ppb was found in water samples from the Rio Grande, possibly because most of the lower Rio Grande Valley drains into the Arroyo Colorado rather than into the Rio Grande.

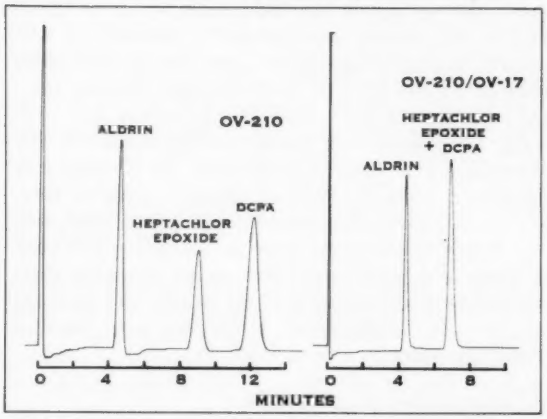

FIGURE 2.—*Gas chromatograms showing differences in elution pattern between OV-210 and OV-210/OV-17 column materials*

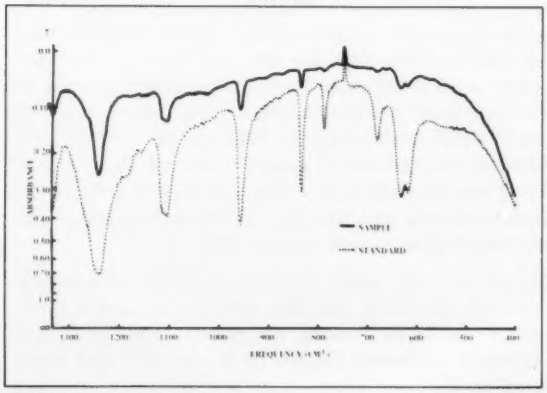

FIGURE 3.—*Infrared spectral comparison of DCPA standard and water sample, showing confirmation of DCPA in arroyo water*

Only one bottom-sediment sample contained a trace (42 ppb) of DCPA. Water samples were analyzed without removing the particulate matter; it is not known whether the DCPA residues are in the water or in association with suspended particles.

MENHADEN

Monthly samples of menhaden did not reveal any DCPA residues until March 1972 (Table 2). Each sample included 25 g of the small fish with the heads and tails removed. Since specific tissues were not analyzed, these results cannot be directly related to the values for other fish. Based on current procedures, however, it appears that the menhaden contain greater amounts of DCPA than the other species of fish sampled to date. The higher residue values probably are not due to contamination from the gut contents because the gut was extracted separately on several occasions and residues were less than those in the remainder of the tissues.

As with the water residue, the amount of DCPA in menhaden varied widely. In November an additional sample taken at a site nearby contained 7.27 ppb DCPA; the regularly scheduled sample had only 0.52 ppb.

OTHER FISH

In May and July 1972, two large samples of speckled trout were collected for chlorinated hydrocarbon analysis. The May collection of 58 females and 8 males averaged 38 cm in length and 500 g in weight. Testing showed that 29 percent of the females and 50 percent of the males in this collection contained DCPA in one or more tissues (Table 3). Overall, the highest levels were found in the liver tissues, although these were not always positive when other tissues were. The greatest amounts recorded for liver, ovaries, testes, and flesh were 196, 85, 200, and 26 ppb, respectively.

The second sample of 9 females and 10 males averaged 38 cm in length and weighed 568 g. DCPA was not present in any of the tissues. No effort was made to determine whether the two samples were from the same fish population. Two speckled trout (427 g and 257 g) collected during March 1973 also had DCPA residues.

Several other species of fish (Table 1) were collected after the initial detection of DCPA in fish. Analysis of one mullet showed residues comparable to those found in menhaden of the same collection. Five samples of Rio Grande perch were collected at various locations along the Arroyo Colorado over a 4-month period. DCPA residues in the flesh varied from 0 to 217 ppb. One redfish had a trace of DCPA in the liver and testes.

TABLE 2.—*DCPA residues in unfiltered water samples and menhaden from Arroyo Colorado near Harlingen, Tex.*

Month	DCPA Residues					
	1971		1972		1973	
	Water, ppb	Fish, ppm	Water, ppb	Fish, ppm	Water, ppb	Fish, ppm
January	0.12	0	0	0	0.59	0.97
February	0.05	0	0.08	0	1.59	—
March	0	0	0	2.78	1.05	2.78
April	0.23	0	0.15	0	0.83	0.22
May	0.10	0	0	0	0.47	0.27
June	0.02	0	0.02	0	0.02	1.07
July	0	0	0	0	0.04	0
August	0	0	0.03	0	0.06	0
September	0	0	0	0.35	0.05	0
October	0	0	1.25	8.15	0.18	0.24
November	10.00	0	0.96	0.52	1.09	0.05
December	0	0	0	1.75	0.55	0

TABLE 3.—*Residues of DCPA in spotted sea trout from Three Islands, Laguna Madre*

Date Collected	Number and Sex of Fish Analyzed	Total Fish with Residues		Tissue Sampled	Fish with Residues		Mean Residue, ppb	
		No.	%		No.	%	Per Sample	Per Positive Sample
May 1972	58 females	17	29	liver	8	14	8	60
				ovaries	14	24	8	36
				flesh	3	5	1	16
	8 males	4	50	liver	4	50	56	112
				testes	2	25	35	28
				flesh	0	0	0	0
July 1972	9 females	0	0	liver				
				ovaries				
				flesh	0	0	0	0
	10 males	0	0	liver				
				testes				
				flesh				
March 1973	1 female	1	—	liver	1	100	1	1
				ovaries	1	100	1	1
				flesh	0	0	0	0
	1 male	1	—	liver	1	100	472	472
				flesh	1	100	55	55

AIR MONITORING

Weekly air monitoring of the lower Rio Grande Valley by alternately sampling the Harlingen and McAllen areas showed no DCPA residues from February to September 1972. Of 17 weekly samples taken from September through December, 11 were positive for DCPA. Routine weekly air sampling was discontinued at the end of December, but three additional samples were taken during 1973. A trace of DCPA was found in early January 1973, but none was detected in samples taken later in January and in March.

As might be anticipated, the residues were found during the first part of the vegetable-growing season and residues were higher in the vegetable-growing area. Mean air sample values for the 4-month period with DCPA residues during the fall was 1.94 $\mu g/m^3$ with extreme values of 0.90 and 8.62 $\mu g/m^3$. Higher levels were found in samples near McAllen, a vegetable-growing area, than in those from Harlingen during the same time period. Mean residues per positive sample were 4.73 and 2.59 $\mu g/m^3$ for McAllen and Harlingen, respectively.

Conclusion

DCPA is a frequent, although intermittent, chlorinated hydrocarbon contaminant of the aquatic and estuarine environments of the lower Rio Grande Valley. How long it persists in these environments is unknown; it is probably very transient, because it is readily broken down by microorganisms in soils (*9*). Hexachlorobenzene (HCB) residues have not been found in environmental samples from this area although the commercial DCPA preparation may have up to 10 percent HCB in some formulations (*10*). The source of aquatic contamination

with DCPA is unknown at this time. It seems unlikely that windblown residues or contamination from a single spill could account for the presence of these residues over this period of time.

To date, DCPA has not been detected in water in amounts that might cause acute intoxication of fish. Investigation is needed, however, to determine subacute toxicity of this compound to fish at low levels. Investigation of the method by which DCPA enters the aquatic ecosystem is also needed.

Acknowledgments

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Degradation of Four Organophosphate Insecticides in Grape Tissues

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ABSTRACT

Four organophosphate pesticides were applied to field-grown grapes at maximum recommended rates for pest control in Fresno County, Calif. Periodic sampling and analyses were carried out for each pesticide immediately before and following application on leaves, bark, cane, fruit, and soil. At harvest time 42 days after the initial application, azinphosmethyl and ethion residues were found on leaves at approximately 20 percent and 2.5 percent, respectively, of their original levels. Phosalone, applied 14 days after the azinphosmethyl and ethion treatment, still had about 30 percent of its original residue at harvest time; naled residues were not detected (<0.10 ppm) 4 days after application. Residues on cane and bark were quite stable and accounted for a relatively high percentage of the total residues found on the plant at harvest. Total extracted residues found on the plant at harvest amounted to approximately 280 ppm.

Introduction

During the summer of 1970, 51 acres of Thompson seedless grapes were set aside in Fresno County, Calif., for the purpose of spraying with four different organophosphate insecticides at rates normally recommended for pest control and within the minimum time period permitted by Federal regulation. Related data on chlorinesterase depressions in farm workers exposed to pesticide residues are reported by Bailey, et al. (*1*). The article at hand deals only with the deposit and degradation of the respective chemicals following application. Very little, if any, published information is available concerning safe reentry times for field workers following application of pesticides on grapes. This study aimed to determine the degradation pattern of the chemical in the various grape tissues in the treated vineyard, and whether a potential problem exists for workers who might be exposed to that chemical. If a possible health problem exists, further studies will be necessary to determine safe reentry times following application.

Methods and Procedures

Four organophosphate pesticides were applied in five replicate plots on 51 acres in Fresno County, Calif., beginning August 27, 1970. One application of each insecticide was applied to each replicate plot on staggered days to see whether different days of application would give appreciably different residue levels. The four pesticides selected were ethion or Nialate[R], azinphosmethyl or Guthion[R], naled or Dibrom[R], and phosalone or Zolone[R]. Some of the more toxic metabolites and degradation products were also analyzed. For example, dichlorvos or DDVP, 2,2-dichlorovinyl dimethyl phosphate, is a degradation product of naled and was thus analyzed with naled. Chemical structures of these pesticides and their oxygen analogs are shown in Figure 1. Table 1 shows the rate and formulation of each insecticide application, and the interval between treatment and harvest. Amounts actually applied were a little higher in the first two applications than those originally planned because of a miscalculation concern-

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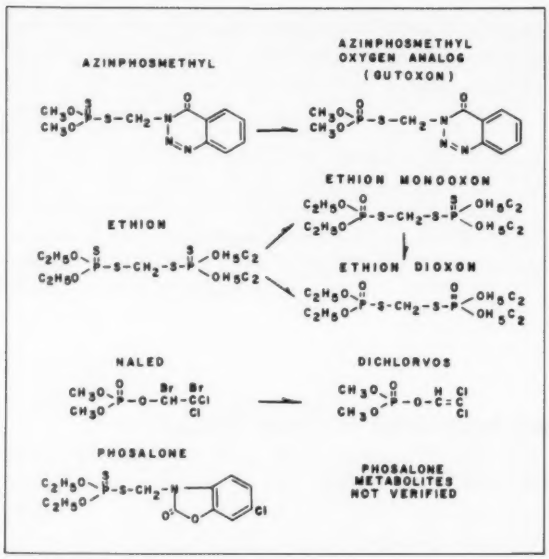

FIGURE 1.—*Chemical structures of azinphosmethyl, ethion, naled, phosalone, and their oxygen analogs*

TABLE 1.—*Insecticides applied to grape fields in Fresno County, Calif., 1970*

Insecticide	Formulation [1]	Active ingredient, lb/acre	Days: application to harvest
Ethion	25% WP	1.60	42
Azinphosmethyl	50% WP	1.90	42
Phosalone	3 lb/gal EC	3.00	28
Naled	8 lb/gal EC	1.00	14

[1] WP: wettable powder.

ing the spraying apparatus. But such an error is not unlike a typical field applciation. Periodic samplings, starting with a pre-application sample, were continued throughout the growing season until harvest. Samples were taken on leaves, bark, cane, fruit, and the surface inch of soil immediately below the plants. In all samplings, random samples were selected from inside and outside each plant.

Following sampling, specimens were immediately placed in a dry-ice chest and kept frozen at subzero temperatures until extracted into an organic solvent. A mixture of 10 percent methanol in chloroform was selected as the final organic solvent. As reported by Bowman and Beroza in 1968 (*2*), this solvent system was superior for extracting the oxygen analogs and degradation products as well as the parent materials for the many organophosphate pesticides studied. Samples of leaves, bark, and cane weighing 100 g were placed in a 2-liter Erlenmeyer flask and shaken with 1,000 ml of solvent on a rotary shaker for 30 minutes. The solvent was filtered through anhydrous sodium sulfate into a glass storage bottle and stored in the dark until analyzed.

Purification of samples was not considered necessary except to analyze ethion and the oxygen analogs. Interfering materials were removed from the ethion extracts by evaporating a portion of extract representing 10 g of crop to dryness, then adding 10 ml of benzene. A chromatographic column was prepared by placing a plug of glass wool into a 2.0-by-11.0-cm borosilicate glass chromatographic tube with a 250-ml reservoir attached to the top. The column was packed with 9 cm of activated florisil and topped with 2 cm of granular anhydrous sodium sulfate, then washed with 80 ml of benzene which was subsequently discarded. The sample was transferred to the column and eluted with 50 ml of 10 percent ethyl ether in benzene. The solvent was evaporated and the residue remaining was transferred with acetone to a McNaught and McKay-Shevky-Stafford sedimentation tube. The sample was then made to a known volume before injecting into the gas chromatograph.

Oxidized analogs of ethion and azinphosmethyl on leaves were also separated from their interfering materials by means of an activated florisil column. Four separate solvent mixtures were added to the column, with an increase in polarity with each subsequent solvent mixture. The first fraction included 200 ml of benzene; this was followed by 100 ml of 25 percent ethyl ether in benzene, 100 ml of 25 percent acetone in benzene, and a fourth and final fraction of 50 ml of acetone. Fractions one through three contained ethion monooxon and the last two fractions contained ethion dioxon, as well as the oxygen analog of azinphosmethyl commonly referred to as gutoxon. Because the third fraction of 25 percent acetone in benzene contained both ethion monooxon and ethion dioxon, the residue analysis of each product was cleaned separately. Metabolites of phosalone were not analyzed routinely because of interfering substances and the inability to isolate specific products. Analyses before and after cleanup were tried with gas-liquid chromatography and thin-layer chromatography (GLC and TLC).

There were no detectable phosphorus compounds besides those already mentioned following the application of phosalone to the treated plots. Thin-layer chromatography, however, did reveal so many cholinesterase-inhibiting spots on the chromatogram that separation and detection from known cholinesterase-inhibiting products was impossible. Since gas chromatography did not give any appreciable residues for the metabolites, it was assumed that the enzyme detection system used for thin-layer chromatography was that much more sensitive than that of gas chromatography. Because GLC measured only the phosphorus element as it passed through the gas-chromatographic column, it was concluded that the relative amount of the metabolites of phosalone was most likely insignificant.

Soil samples were handled somewhat differently than bark, cane, and leaf samples. They were moistened with water only until the soil became glossy, and were shaken on a rotary shaker for 30 minutes with the extracting solvent. The solvent was filtered through anhydrous sodium sulfate and handled in accordance with the other samples of bark, cane, and leaves. All fruit samples were analyzed for total residue by chopping the frozen grapes in a Hobart food chopper, transferring 100 g into a blender with 400 ml of stripping solvent, blending for 2 minutes, and shaking for 30 minutes. The organic solvent containing the residue was then filtered through anhydrous sodium sulfate into a glass storage bottle.

All extracts except gutoxon were analyzed on a gas chromatograph; most were analyzed at least twice using two different detectors. Recovery samples were also run on each date, and replicates were sampled from the control plots for each pesticide applied. Fortified recovery studies for each organophosphate pesticide were made at the time of extraction and just before analysis at the 1 ppm level.

The two detectors and gas chromatographs used throughout the study included a Varian Aerograph Model 204 gas chromatograph equipped with a cesium bromide thermionic flame detector (*3,4*) and a Micro-Tek Model MT 220 gas chromatograph equipped with a dual flame photometric detector (*5,6*). Both GLC systems are relatively specific for phosphorous-containing organic compounds. A chlorinesterase detection method (*7*) was also utilized following separation by thin-layer chromatography for detecting and confirming the presence of naled, dichlorvos, ethion monooxon, ethion dioxon, and gutoxon; this method was used with the gas chromatography procedure. Gutoxon was the only metabolite that was not analyzed by GLC. For this particular compound, only thin-layer chromatography was used. The developing solvent was benzene: ethyl acetate: methyl alcohol, 90:8:2.

The GLC columns used throughout the study were: a 6-ft-by-⅛-in.-o.d. borosilicate glass packed with 10 percent DC 200 on 100/120-mesh Gas Chrom Q; a 6-ft-by-¼-in.-o.d. borosilicate glass packed with 3 percent OV-225 on 60/80-mesh Gas Chrom Q; a 3½-ft-by-¼-in.-o.d. borosilicate glass packed with 5 percent OV-225 on 60/80-mesh Gas Chrom Q; and a 6-ft-by-¼-in.-o.d. borosilicate glass packed with 10 percent OV-17 on 60/80-mesh Gas Chrom Q. The first two columns were used for separating and analyzing ethion, azinphosmethyl, and phosalone. The third column was used to separate naled and dichlorvos; the fourth column was used to separate ethion monooxon and ethion dioxon. Temperatures of the injection part and detector for the Micro-Tek gas chromatograph were 240°C and 290°C, respectively. Injection and detection temperatures for the Varian Aerograph were 227°C and 230°C, respectively. Flow rates of the oxygen, air, hydrogen, and nitrogen carrier gases for the Micro-Tek were 20, 20, 200, and 90 ml/min, respectively. The Varian Aerograph flow rates of the air, hydrogen, and nitrogen (carrier) gases were 170, 16.5, and 20 ml/min, respectively. Column temperatures for both instruments for ethion, azinphosmethyl, and phosalone were held to 230°C; ethion monooxon and ethion dioxon samples were operated at 245°C. The naled and dichlorvos analyses were handled differently in that the column temperature was programmed with an initial temperature of 130°C for 2 minutes following injection. Then it was programmed at a rate of 30°C/min until a final temperature of 170°C was reached; it was held at that temperature for 6 minutes before cooling to the initial temperature of 130°C.

Results and Discussion

Several extraction solvents including acetonitrile, benzene, chloroform, and 10 percent methanol in chloroform were compared for the quantity of residue extracted and the variability of the residue between individual samples. The quantity of the residue in the substrates was about the same regardless of the solvent selected; however, the 10 percent methanol in chloroform gave results slightly more consistent than those produced by the other solvents. Extraction time of 30 minutes was also compared with another procedure (*8*) which included shaking the contents in 1:2 ratio of crop tissue to solvent for 2 minutes, letting it rest for 2 minutes, shaking for ½ minute, and resting ½ minute before transferring extract into a sample bottle. Again, residue levels were similar and well within the variations found between the replicates. However, there was a considerable degree of inconsistency between these samples and those produced by the 30-minute extraction period.

This study was concerned with maximum levels of residue that might be associated with surface exposure and includes residue levels that might be incorporated into the plant surface, as in the plant waxes or tissues just under the immediate surface. Therefore, residue levels reported here are probably higher than the actual surface residue to which a worker would be exposed in a field situation. Ascertaining how an individual is exposed to pesticides and which pesticides he is exposed to is very difficult. Much work needs to be done in this regard, but this was not the objective of the present study.

Authors of this study had anticipated looking at some of the toxic degradation products which are more polar than their parent compounds. Therefore, it was necessary to extract with a mixture including a polar

solvent. Ten percent methanol in chloroform (2) was used throughout the study.

Results from the fortified recovery studies ranged from 95 to 100 percent for all pesticides analyzed including the degradation products. The only two exceptions to this were ethion dioxon, which ranged from 80 to 95 percent recovery, and gutoxon, which could not be checked for a specific recovery because it was analyzed by thin-layer chromatography. Fortified extracts of gutoxon did look satisfactory even when fortified at 0.1 ppm. However, this was only a visual observation and quantitation was not attempted.

Soil, leaves, cane, and bark were sampled periodically following each insecticide application. Harvested grapes were also sampled. Figures 2-4 show in graphic form the residue levels found on the various tissue samples, except for soil samples. Residue levels in the soil were relatively low, ranging from about 3.0 ppm on the first day of application to 1.0 ppm at harvest for azinphosmethyl and phosalone. Ethion had about the same initial residue but degraded rather rapidly, with a final residue at 42 days of 0.15 ppm. Naled had an initial level of about 0.5 ppm; the second sampling four days later showed less than 0.1 ppm except for one sample which had 0.2 ppm. Fruit samples of grapes were harvested and analyzed on two different dates, September 28 and October 8. Table 2 shows these residues and residues found on all tissues sampled at time of harvest. In all cases, residues found on the fruit were within the U.S. Government tolerance for each of the four pesticides surveyed.

As mentioned earlier, insecticides were not applied to the five replicate plots (R1-5) on the same day. The plot identified as R1 had its first application of ethion and azinphosmethyl on August 27, R2 and R3 on August 28, and R4 and R5 on August 31. The variability of residue between the three dates of application was less than the variability between samples with the same application on the same plot sampled the same day. Therefore, factors attributed to variation of application days were generally considered insignificant. The same was true of phosalone which was applied to R1, R2, and R3 plots on September 11. R4 and R5 plots received their first application of phosalone on September 14. Naled was applied to all five plots on September 24.

For each periodic sampling, Figures 2-5 show the highest, lowest, and average residue for each pesticide per

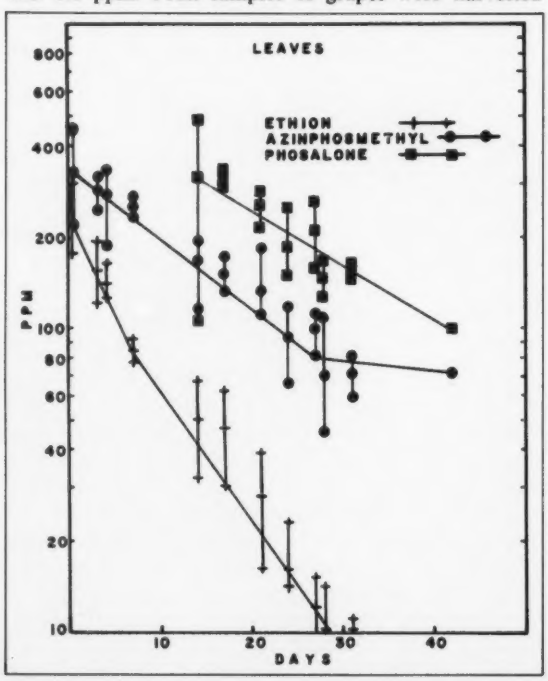

FIGURE 2.—*Residues of ethion, azinphosmethyl, and phosalone extracted from grape leaves with an organic solvent wash from samples taken at time of application and throughout growing season. Each group of points represents range and mean of residues in samples after application*

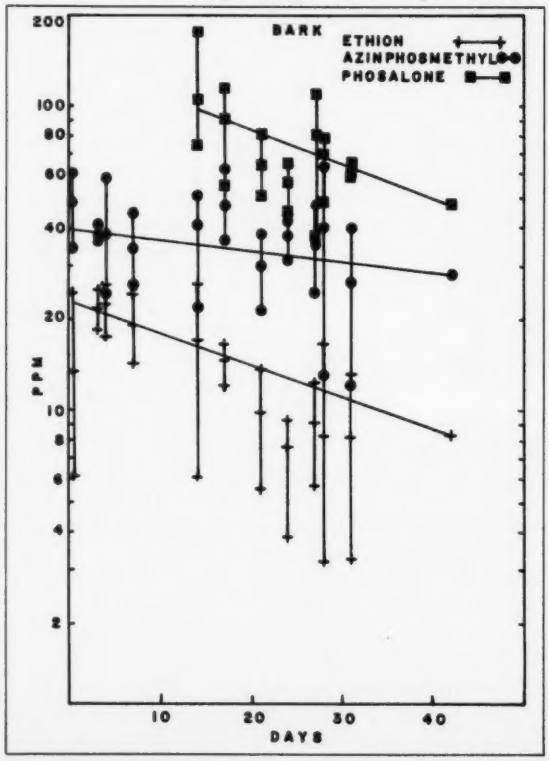

FIGURE 3.—*Residues of ethion, azinphosmethyl, and phosalone extracted from grape bark with an organic solvent wash from samples taken at time of application and throughout growing season. Each group of points represents range and mean of residues in samples after application*

TABLE 2.—*Insecticide residues found on grape tissues at time of harvest*

Insecticide	Tissue Analyzed	Residues Found, ppm
Azinphosmethyl	Leaves	71.3
	Bark	28.2
	Cane	6.60
	Fruit	2.56 [1]
	Soil	1.00
Ethion	Leaves	5.80
	Bark	8.20
	Cane	0.40
	Fruit	0.70 [1]
	Soil	0.15
Phosalone	Leaves	98.8
	Bark	47.0
	Cane	6.80
	Fruit	1.08 [1]
	Soil	1.00
Naled	Leaves	<0.10
	Bark	2.20
	Cane	<0.10
	Fruit	<0.10 [1]
	Soil	<0.10

[1] Represents average of residues taken on two days of harvest: Sept. 28 and Oct. 8.

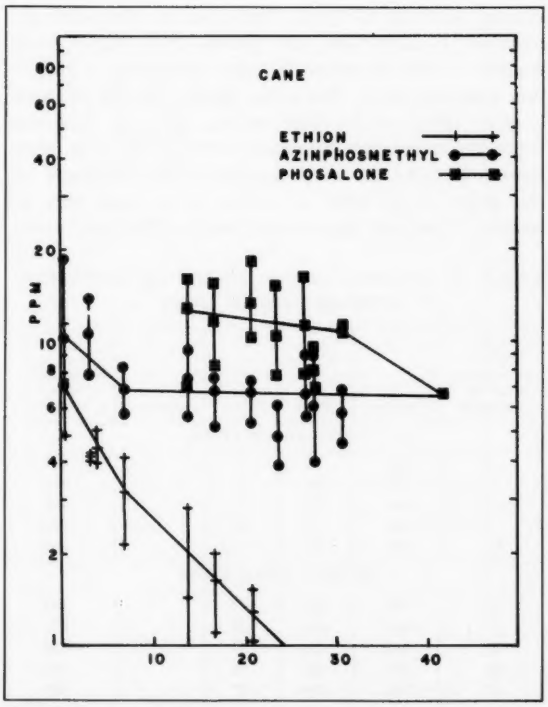

FIGURE 4.—*Residues of ethion, azinphosmethyl, and phosalone extracted from grape cane with an organic solvent wash from samples taken at time of application and throughout growing season. Each group of points represents range and mean of residues in samples after application.*

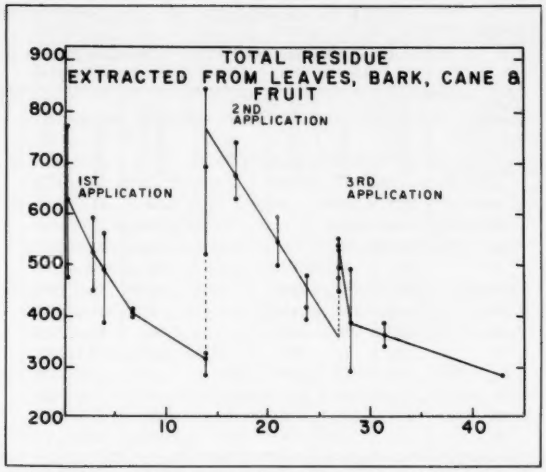

FIGURE 5.—*Total insecticide residue extracted from leaves, bark, cane, and fruit of grape*

period from the initial time of the azinphosmethyl and ethion application. All reported naled residues include dichlorvos, even though the two insecticides were analyzed individually. Table 3 shows the average levels of residue found on the four separate substrates from initial day of application until harvest. Even though residue levels among samples varied considerably, levels in relation to time followed first-order kinetics, particularly in leaf tissues. Variability was much greater between cane and bark samples than between soil and leaf samples. However, data did indicate that the rate of decline of each pesticide was considerably slower after the first few days, particularly for azinphosmethyl and phosalone. The irregularity between samples in the corky tissues can be explained by irregular absorption and adsorption and by exposure of the tissue to the spray. Irregularity is also explained by the variable exposure of the tissue to sunlight and other degradation processes. It is difficult to get complete random sampling of old and new tissues from each plant because of the irregularities mentioned which may also explain the variability between samples.

In all cases, the stability of the insecticides in the bark and cane was very significant. Even 42 days after application, about half the original residue remained with the exceptions of naled and ethion. Naled was not detected in the cane; however, it was detected in the bark 14 days after application. Even though the residue level was relatively small, with about 15 percent of the initial deposit remaining at harvest time, the rate of decay was only 50 percent from day 4 to day 15. It appears that once the pesticide is in contact with the corklike tissue, it is absorbed readily and becomes quite stable over a long period of time. This may or may not be significant in relation to the exposure of field

TABLE 3.—*Degradation of azinphosmethyl, ethion, phosalone, and naled in grape plant tissues*

Days: initial application (day 0) to harvest	Average Residue on Leaves, ppm				Average Residue on Bark, ppm				Average Residue on Cane, ppm				Average Residue on Soil, ppm			
	Azinphos-methyl	Ethion	Phosalone	Naled	Azinphos-methyl	Ethion	Phosalone	Naled	Azinphos-methyl	Ethion	Phosalone	Naled	Azinphos-methyl	Ethion	Phosalone	Naled
0	322	225			48	13			10.4	7.5			2.8	1.4		
3	283	156			38	22			10.6	4.0			1.6	1.4		
4	271	139			38	22			8.1	4.5			3.7	2.4		
7	251	84			34	19			6.9	3.1			3.1	1.5		
14	167	49	310		41	17	106		7.5	1.4	13		1.5	0.56	2.9	
17	150	37	310		47	15	92		6.8	1.6	12		2.3	0.44	3.5	
21	132	28	252		30	10	65		6.9	1.3	13		1.1	0.20	2.5	
24	92	16	182		37	7.6	55		4.8	0.85	10		1.3	0.35	1.7	
27	98	12	210	36	35	8.9	80	14	6.6	0.90	11	1.1	1.6	0.28	2.1	0.27
28	69	10	144	<0.1	40	8.2	70	8.2	6.0	0.70	8.0	<0.10	1.7	0.40	1.8	<0.10
31	70	10	156	<0.1	26	8.2	62	5.6	5.7	0.60	10.5	<0.10	1.5	0.26	2.0	<0.10
42	71	5.8	99	<0.1	28	8.2	47	2.2	6.6	0.40	6.8	<0.10	1.0	0.15	1.0	<0.10

workers. Because high levels of residue are involved, however, the matter needs to be investigated.

The two oxygen analogs of ethion on grape leaves are shown in Table 4. All replicate samples analyzed for the metabolites were composited according to days from application. Residues of ethion monooxon and ethion dioxon were much lower than their parent compound the first few days following application. However, the relative amount of residues for the two products was much higher following the third day. The total amount of both ethion monooxon and ethion dioxon from the fourteenth day after application was approximately 50 percent of its parent compound and continued in this proportion throughout the growing season. Even though the percentage of these metabolites was quite high, the total amount of residue was much lower than other organophosphate pesticides studied. Gutoxon, the oxygen analog of azinphosmethyl, was analyzed only the first 14 days following initial application due to the background on the thin-layer plates following the phosalone application. None of the samples analyzed showed any detectable residues of gutoxon or other cholinesterase-inhibiting products in excess of 1 ppm for this period.

TABLE 4.—*Degradation of ethion, ethion monooxon, and ethion dioxon on grape leaves*

Days: initial application (day 0) to harvest	Residue on leaves, ppm			
	Ethion	Ethion Monooxon	Ethion Dioxon	Ethion Monooxon + Ethion Dioxon
0	225	25	2.0	27
3	156	15	2.6	17.6
7	84	19	13	32
14	49	10	15	25
17	37	7.2	8.8	16
21	28	6.3	4.5	10.8
24	16	4.6	3.6	8.2
27	12	3.4	3.4	6.8
31	10	2.6	1.8	4.4
42	5.8	1.3	1.4	2.7

Table 5 and Figure 5 show the total residue less the metabolites on the leaves, bark, cane, and fruit from initial application until harvest. The day azinphosmethyl and ethion were applied, the average residue level was 626 ppm and after 14 days the sum of the residue in the plant dropped to about 300 ppm. On this day, phosalone was applied and the total residue rose to an average level of 688 ppm. Just prior to the naled application 14 days later, the residue level should have reached a level of about 350 ppm, according to projection from the graph. The naled application did not have a great effect on the total residue after the first day following application because of its rapid decay in most tissues. Perhaps the most significant fact displayed by this graph is the level of residue at or near time of harvest. This level approached nearly 300 ppm, about

TABLE 5. *Averages (ppm) of all four insecticides from initial application to harvest*

Days: initial application (day 0) to harvest	Leaves	Bark	Cane	Fruit	Total Application
FIRST APPLICATION					
0	547	61	18		626
3	439	60	15		514
4	410	60	13		483
7	335	53	10		398
14	241	56	9.3		306
SECOND APPLICATION					
14	501	166	21		688
17	497	154	20		671
21	412	105	21		538
24	290	100	16		406
27	350	119	17		486
THIRD APPLICATION					
27	328	144	22		494
28	223	126	15	12	376
31	236	102	17	14	369
42	175	85	14	4.4	278

one half the initial and total deposit of azinphosmethyl and ethion, and one third the highest residue level found on any one sample at any given time. Even though this residue level is high, it likely does not reflect the total measure of hazard to which an agricultural worker is exposed because a considerable portion of these materials were found in the bark and cane and, as mentioned previously, may not present an immediate hazard. This aspect of pesticide residue in such tissues needs to be considered in a worker-safety study. Studies should also be conducted to ascertain whether the high residues found in corklike tissue are, like leaf tissues, a potential hazard and, if so, in what manner and to what degree an individual would be exposed to them.

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APPENDIX

Chemical Names of Compounds Discussed in This Issue

ALDRIN	Not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
AROCLOR 1260	PCB, approximately 60% chlorine
AZINPHOSMETHYL (Guthion®)	*0,0*-dimethyl *S*[4-oxo-1,2,3-benzotriazin-3(4H)ylmethyl] phosphorodithioate
BHC (Benzene Hexachloride)	1,2,3,4,5,6-hexachlorocyclohexane (mixture of isomers). Commercial product contains several isomers of which *gamma* is most active as an insecticide.
CHLORDANE	1,2,3,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene. The technical product is a mixture of several compounds, including heptachlor, chlordene, and two isomeric forms of chlordane.
DCPA (Dacthal®)	Dimethyl-tetrachloroterephthalate
DDD	See TDE.
DDE	Dichlorodiphenyl dichloro ethylene. (Degradation product of DDT.) Main component: 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene *o,p'*-DDE 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethylene *p,p'*-DDE 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene
DDMU	(1-chloro-2,2-bis[*p*-chlorophenyl]ethylene)
DDT	*α*-bis(*p*-chlorophenyl)*B,B,B*-trichloroethane. Numerous isomers in addition to *p,p'*-DDT are possible, and some are present in the commercial product. *o,p'*-DDT [1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane]
DDVP	See dichlorvos.
DIBROM	See naled.
DICHLORVOS	2,2-dichlorovinyl dimethyl phosphate
DIELDRIN	Not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethano=naphthalene
ENDRIN	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
ETHION	0,0,0',0'-tetraethyl S,S'-methylene bisphosphorodithioate
GUTHION	See azinphosmethyl.
HEPTACHLOR	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-*endo*-methanoindene
HEPTACHLOR EPOXIDE	1,4,5,6,7,8,8-heptachloro 2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane
LINDANE	Gamma isomer of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) of 99+% purity
METHOXYCHLOR	1.1,1-trichloro-2,2-bis(*p*-methoxyphenyl) ethane
MIREX	Dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta[cd]pentalene
NALED	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
NONACHLOR	Component of commercial quality chlordane
PHOSALONE	0,0-diethyl S-(6-chloro-2-oxo-benzoxazolin-3-yl) methyl phosphorodithioate
POLYCHLORINATED BIPHENYLS (PCB's)	Mixtures of chlorinated biphenyl compounds having various percentages of chlorine
RESMETHRIN	(5-benzyl-3-furyl methyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate
TDE (DDD)	2,2-Bis (*p*-chlorophenyl)-1,1-dichloroethane (including isomers and dehydrochlorination products)
TOXAPHENE	Chlorinated camphene (67-69% chlorine); product is a mixture of polychlor bicyclic terpenes with chlorinated camphenes predominating
ZOLONE	See phosalone.

ERRATA

PESTICIDES MONITORING JOURNAL, Volume 7, Number 1. In the paper "Pesticide Residues in Natural Fish Populations of the Smoky Hill River of Western Kansas—1967-69," page 56, right column, third paragraph, second sentence, should read, "Dieldrin was detected in a small percentage (15%) of the samples; most of these had only trace (<0.01 ppm) amounts."

In Table 4 of the same paper, sample numbers and dieldrin residues for Station 5 were misquoted. Correct values for Station 5 appear below:

TABLE 4.—*Pesticide residues in fish and fish tissues from the Smoky Hill River, Kansas—1967-69*—Continued
[T = <0.01 PPM]

Species	Tissue	Number of Samples [1]	Number of Times Detected and Range ( ) in PPM of Detected Residues [2,3]					
			Dieldrin	Heptachlor Epoxide	*o,p'*- DDT	*p,p'*- DDT	*p,p'*- DDE	*p,p'*- DDD
STATION 5								
Stoneroller	Whole body	8	3 (T-.01)	0	0	0	8 (T-.09)	0
Red shiner	Whole body	9	3 (T-.01)	0	0	0	8 (.01-.04)	0
Plains killifish	Whole body	7	1 (T)	0	0	0	4 (T-.01)	0
Green sunfish	Whole body	6	2 (T-.03)	0	0	1 (.02)	5 (T-.03)	0
Carp	Flesh	4	0	0	1 (.07)	0	2 (.01)	0
River carpsucker	Flesh	2	1 (T)	0	0	0	1 (.01)	0
Channel catfish	Flesh	6	1 (T)	0	1 (.07)	0	5 (T-.02)	1 (.02)
Carp	Testes	4	1 (T)	0	0	0	4 (.01-.07)	0
	Ovaries	4	0	0	0	0	3 (.01-.02)	0
River carpsucker	Testes	1	1 (T)	0	0	0	1 (.01)	0
	Ovaries	1	1 (T)	0	0	0	1 (.01)	0

[1] For whole body analysis, individuals of one species from one station were pooled to make one sample. The sample of flesh consisted of a pooling of equal weights of flesh taken from each individual of one species; gonads were treated similarly, keeping sexes separate.
[2] Wet-weight basis.
[3] Endrin, aldrin, and heptachlor residues were all zero values.

PESTICIDES MONITORING JOURNAL, Volume 7, Number 3/4. In the paper "Surveys of Mercury Levels in Fish and Other Foods," page 131, left column, last line, should read "ppm total mercury in the whole fish."

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The PESTICIDES MONITORING JOURNAL welcomes from all sources qualified data and interpretive information which contribute to the understanding and evaluation of pesticides and their residues in relation to man and his environment.

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